



National Health and Environmental Effects Research Laboratory

An Annual Report
of Accomplishments
for Fiscal Year 2000

November 2000

National Health and Environmental Effects Research Laboratory

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U.S. Environmental Protection Agency
Office of Research and Development
National Health and Environmental Effects Research Laboratory
Research Triangle Park, NC 27711



notice

The U. S. Environmental Protection Agency through its Office of Research and Development conducted and managed the research described in this report. It has been subjected to the Agency's peer and administrative review and has been approved for publication as an EPA document.

abstract

This Annual Report showcases some of the research activities of the National Health and Environmental Effects Research Laboratory (NHEERL) in various health and environmental effects research areas. The report is an indicator of progress and accomplishments that NHEERL has made in Fiscal Year 2000 to meet the requirements of the Government Performance and Results Act (GPRA). NHEERL's highlighted research is organized by these goals. Specific research areas included for this year are: (1) Particulate Matter, (2) Air Toxics, (3) Drinking Water, (4) Aquatic Stressors, (5) Pesticide Residues in Food; (6) Pesticides in the Environment; (7) Global Climate Change, (8) Ecosystems Protection, (9) Human Health Protection, and (10) Endocrine Disruptors.

letter from the director

Scientists at ORD's National Health and Environmental Effects Research Laboratory (NHEERL), are charged with determining the impacts of environmental stressors on human and ecosystem health, the degree to which those stressors cause harm, and the many factors, both internal and external, that influence the degree of harm. Our research agenda is driven by the need either to provide scientific information to inform specific regulatory decisions or to resolve uncertainties in the risk assessment process and characterize the state of the environment.

This Annual Report highlights some of NHEERL's research accomplishments which address key human health and environmental effects issues. In addition, the report demonstrates the progress that NHEERL has made in meeting the requirements of the Government Performance and Results Act (GPRA). The highlighted accomplishments are presented by the strategic goals contained in the Agency's strategic plan. These include: (1) Clean Air (Particulate Matter and Air Toxics); (2) Clean Water (Safe Drinking Water and Aquatic Stressors); (3) Food Quality Protection - Safe Food; (4) Safe Communities; (5) Climate Change; and (6) Sound Science. Through a program of integrated multi-disciplinary research, NHEERL scientists have addressed key scientific questions facing the Agency in these high priority environmental areas. For example, why do elevated exposure levels affect some populations more than others? How do pesticide residues on foods affect human health? How will global climate changes affect ecosystems along coastal regions?

Using a risk-based approach, our research provides EPA the scientific data needed to inform policies and regulatory programs that protect human health and the environment. We recognize the critical and complex nature of our research and its role in contributing to the on-going effort to safeguard human health and the natural environment. We are proud to share some of our most important findings with you.

Lawrence W. Reiter, Ph.D.
Director, National Health and Environmental Effects Laboratory
Office of Research and Development
Research Triangle Park, North Carolina

table of contents

1

introduction

5

particulate matter

11

air toxics

17

drinking water

23

aquatic stressors

29

pesticide residues in food

33

pesticides in the environment

37

global climate change

43

ecosystems protection

49

human health protection

55

endocrine disruptors

Food Quality Protection

National Health and Environmental Effects Research Laboratory

Sound Science

Safe Communities

Advancing Knowledge For a Purpose:

Deciphering the Link Between

Environmental Stressors and their Effects

Climate Change on Human Health and Ecosystems

Highlights of Accomplishments Made During Fiscal Year 2000

The National Health and Environmental Effects Research Laboratory (NHEERL) is an important arm of the U.S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD). NHEERL is EPA's focal point for scientific research on the adverse effects of pollution and other stressors on human health and ecosystem vitality. Our scientists provide information essential to effective risk assessment, which is the scientific basis for regulatory and policy decisions.

NHEERL provides vital leadership in both the national and international research communities. Based in Research Triangle Park, NC, NHEERL has nine

divisions in six states and a work force of over 700 federal employees. Five health divisions are centrally located in Research Triangle Park and Chapel Hill, NC, and four ecology divisions are based in ecologically significant regions (Atlantic seaboard, Pacific coast, Great Lakes, and the Gulf of Mexico) to address national and regional ecological risk assessment issues.

Our scientists conduct in-house research as well as participate in collaborative studies with academia, state governments, other federal agencies, and other research organizations around the world. NHEERL research undergoes the highest levels of technical review and

A man with a beard and sunglasses, wearing a blue and white plaid shirt, is standing in a marshy field. He is holding a long, thin white tube that leads into a black, box-like device with various wires and components. The device is resting on the ground. In the background, there is a body of water with a small white sailboat visible on the horizon. The overall scene is outdoors in a natural, wetland environment.

NHEERL research centers are located in several geographically important areas around the nation.

Newport, OR

Duluth, MN

Corvallis, OR

Narragansett, RI

Grosse Ile, MI

Research Triangle Park, NC

Chapel Hill, NC

Gulf Breeze, FL

scrutiny, and our results are published in peer-reviewed journals, reports, and other media as a means of communicating our scientific progress and accomplishments to the public and scientific community. NHEERL scientists also regularly present research findings at symposia, hold membership and leadership positions on scientific committees and workgroups, and participate in various nationally and internationally recognized environmental organizations.

In researching health and ecological risk, NHEERL's structure enables scientists to develop innovative methods and solutions to complex problems in an integrated manner. For example, our focus is not necessarily pollutant-specific. Data extrapolated from both animal and human studies are incorporated into computer models that are used in real-world applications. The combination of a scientifically diverse workforce and highly specialized facilities enables

NHEERL to stay on the cutting edge of health and environmental effects research. Currently, major research activities are focused on the harmful effects of particulate matter and endocrine-disrupting chemicals, and some of our most important projects are described and showcased in this report. It is important to note that this report is not a comprehensive summary of all research completed at NHEERL during this year, but rather, it highlights some of our recent accomplishments in the following areas:

- health effects of airborne particulate matter
- mechanisms of toxicity of air pollutants
- advances in drinking water safety research
- susceptibility of young individuals to certain pesticides
- influence of pesticides on the developing immune system
- effects of global climate change on ecosystems and human populations
- assessment of the condition of aquatic and terrestrial ecosystems
- influence of genetic factors and prior exposures on sensitivity to pollutants

The largest research center within EPA's Office of Research and Development, NHEERL has nine divisions. NHEERL headquarters and five health research divisions are located in Research Triangle Park and Chapel Hill, North Carolina. Four ecology research divisions are located in ecologically significant areas around the country.

NHEERL Health Research Divisions

- Environmental Carcinogenesis (RTP, NC). Studies the association between environmental contaminants and cancer.
- Experimental Toxicology (RTP, NC). Examines the toxicity of environmental contaminants to specific organ systems and bodily functions.
- Human Studies (Chapel Hill, NC). Conducts epidemiologic and clinical research on the human response to environmental contaminants.
- Neurotoxicology (RTP, NC). Studies the effects of chemical and physical agents on the nervous system.
- Reproductive Toxicology (RTP, NC). Develops methods used to study the reproductive and developmental effects of environmental contaminants.

NHEERL Ecology Research Divisions

The ecology research divisions assess the condition of regional ecosystems—including terrestrial and aquatic environments—and study the effects of pollution and other stressors on these ecosystems.

- Atlantic Ecology (Narragansett, RI). Atlantic seaboard ecosystems.
- Gulf Ecology (Gulf Breeze, FL). Gulf of Mexico ecosystems.
- Mid-Continent Ecology (Duluth, MN and Grosse Ile, MI). Inland and freshwater ecosystems.
- Western Ecology (Corvallis and Newport, OR). Pacific coast ecosystems.



The Clean Air Act requires EPA to review the public health risks of particulate matter and the other criteria pollutants every five years. If warranted by this review, the National Ambient Air Quality Standards (NAAQS) are revised. Based on new epidemiologic evidence of increased illness and death associated with particulate matter, EPA revised the NAAQS for particulate matter (PM) in 1997. EPA established new standards for fine particles, PM_{2.5}, particles 2.5 µm in diameter or smaller, and revised existing standards for PM₁₀, particles smaller than 10 µm.¹

Baltimore has air quality representative of urban areas in the eastern United States. Previous epidemiologic studies found a correlation between increased particulate matter levels in the air and hospital admissions due to heart conditions, but no mechanism of action was suggested. Pollution levels, including the fine (PM_{2.5}) and coarse (PM_{2.5-20}) fractions and other gaseous pollutants, were measured inside and outside the retirement facility. Cardiac function was assessed by measuring beat-to-beat heart rate variability, a factor associated with autonomic nervous system control of the heart.

Clean Air particulate matter

Scientists at NHEERL are world leaders in particulate matter research. NHEERL's PM research program is multidisciplinary and includes three major categories of studies: epidemiologic studies of humans, clinical studies of humans, and laboratory studies using tissue cultures and animal models. NHEERL's research is designed to discover

- who is affected by particulate matter exposure.
- how people are affected by particulate matter.
- the physiologic and molecular mechanisms by which particulate matter causes toxicity.
- the toxic components of particulate matter.

EPIDEMIOLOGIC STUDIES

In cooperation with scientists at the University of North Carolina at Chapel Hill, NHEERL researchers studied the effects of ambient air PM on cardiac function in a retirement center in Baltimore, Maryland.

Previous studies have associated *reduced* heart rate variability with sudden cardiac death, increased risk of developing coronary artery disease, and increased risk of death from all causes among survivors of heart attacks. In the Baltimore study, increased ambient PM_{2.5} levels were associated with decreased heart rate variability. The association was more pronounced in individuals with pre-existing cardiovascular conditions. Although this study did not allow researchers to conclude that day-to-day PM_{2.5} variations are definitely associated with day-to-day risk of cardiovascular disease, this was the first study to relate daily variations in particulate matter levels with cardiac autonomic control. The results suggest one potential mechanism for the association between

increased particulate matter levels and hospital admissions for cardiovascular conditions. A similar study is under way in Fresno, California, a community with air quality representative of urban areas in the western United States.

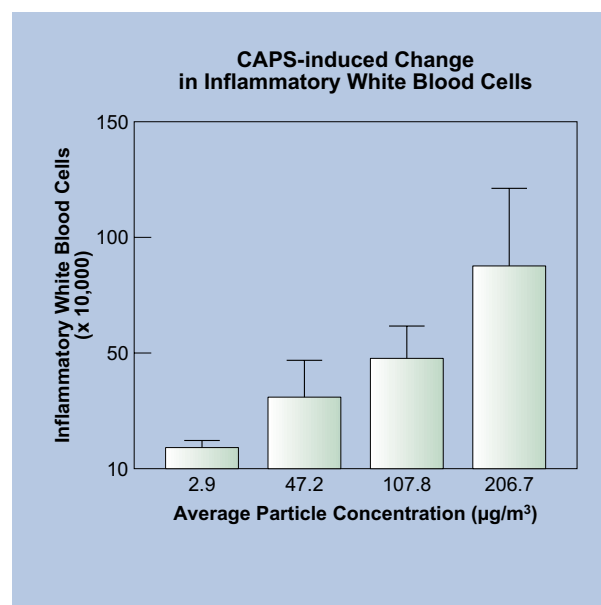
CLINICAL STUDIES

Although epidemiologic studies have consistently demonstrated associations between elevated ambient particulate matter levels and increased morbidity and mortality, the pathophysiological mechanisms underlying these adverse health effects have not been well characterized. Also, the specific effects of particulate matter on susceptible subpopulations have not been well characterized. To address these issues, NHEERL researchers study the health effects of air pollution on human volunteers. Under constant medical supervision, volunteers are exposed to known amounts of concentrated ambient air particles (CAPS) in a state-of-the-science exposure chamber.



NHEERL scientist monitors a volunteer who is being exposed to concentrated ambient air particles (CAPS).

Studying healthy young adults, NHEERL scientists found that particulate matter levels equivalent to those found in many major metropolitan areas could cause mild lung inflammation and could increase factors associated with blood clotting. Supporting the

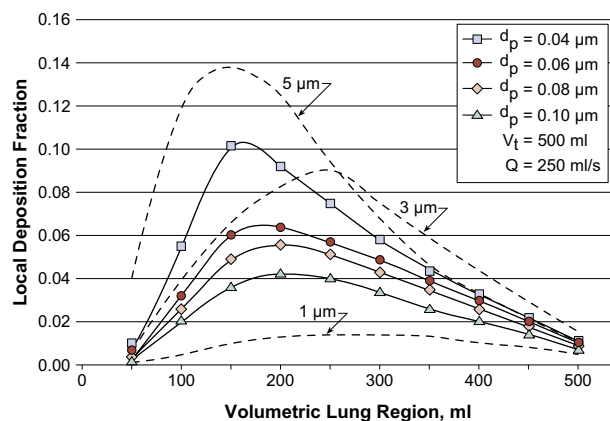


In the lung, inflammatory white blood cells in bronchoalveolar lavage fluid increase as concentrated ambient air particle (CAPS) concentration increases.

findings of the epidemiologic study in Baltimore, healthy elderly volunteers exposed to CAPS experienced decreased heart rate variability. Ongoing CAPS exposure studies are being conducted on potentially susceptible populations, including volunteers suffering from asthma or chronic obstructive pulmonary disease (COPD).

Other scientists at NHEERL are studying the distribution of inhaled particles in the lungs. Because inhaled particles deposit variably in different

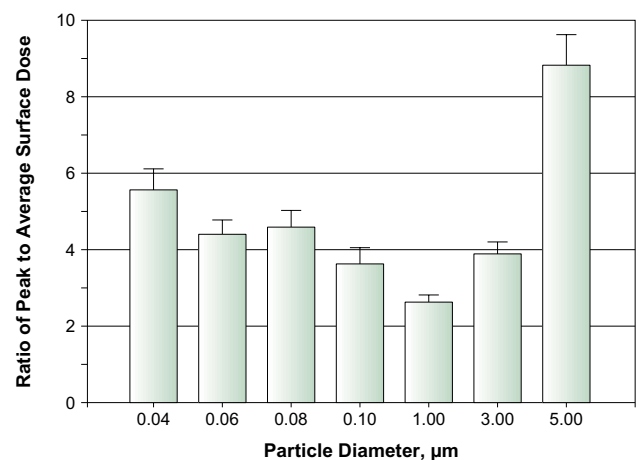
regions of the lung, some areas may receive a dose large enough to cause harm, even though particle concentration in the air may be relatively low. Local regions of the lung that receive greater doses are likely to be affected first. Localized damage may lead to subsequent health effects. NHEERL researchers have studied the deposition patterns of ultrafine particles (less than $0.10\text{ }\mu\text{m}$ in diameter) in healthy young adults and older adults. Interestingly, they found that ultrafine particles resemble coarse particles ($3\text{ to }5\text{ }\mu\text{m}$) in their deposition pattern. Dose per unit surface area of the lung is largest in the most proximal lung regions and decreases with an increase in lung depth. Also, peak surface dose in local areas is 3 to 9 times greater than the calculated average lung dose. These and other results suggest that the localized dose may be a crucial factor in lung injury and subsequent health effects, and that peak surface dose may be a useful measure for estimating potential health hazards of ambient particulate matter.



In the lung, local deposition of inhaled particles increases with increasing lung volume up to a point, after which deposition decreases with increasing lung volume and depth. Also, the deposition pattern of ultrafine particles ($< 0.10\text{ }\mu\text{m}$ in diameter) resembles that of coarse particles ($3\text{ to }5\text{ }\mu\text{m}$ in diameter).
 V_t = tidal volume; Q = respiratory flow rate; d_p = particle diameter.

NHEERL Scientists in Mexico

NHEERL scientists collaborated with Mexican researchers to study the respiratory health of children and adolescents who were lifelong residents of Mexico City, one of the most heavily polluted cities in the world. Scientists and public health officials are particularly concerned about the chronic effects of high levels of air pollution on children, whose lungs are still developing. For this study, a comparison group of children was selected from a low-pollution Mexican city. All participants had no recent history of respiratory illness or prior exposure to tobacco smoke. Every participant from Mexico City experienced upper and/or lower respiratory symptoms, including nosebleeds, nasal dryness and crusting, cough, shortness of breath, and chest discomfort. However, none of the children in the comparison group reported any of these symptoms. Participants with the lowest exposure to outdoor air had the fewest respiratory symptoms. Some of the Mexico City children had evidence of lung hyperinflation, which is suggestive of small airway disease.



For each size of particle studied, peak local deposition dose is many times greater than the average surface dose.

LABORATORY STUDIES

A labor strike that closed a steel mill in the Utah Valley for a year provided opportunities to evaluate the toxicity of ambient air particles. Previous epidemiologic studies found a reduction in hospital

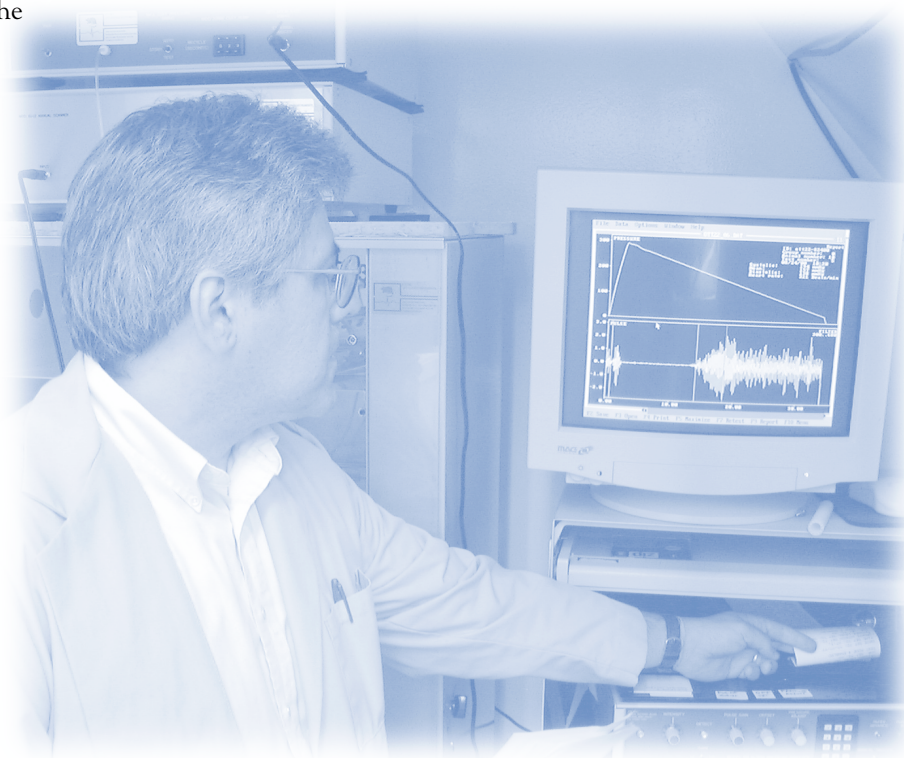


Smog in the Utah Valley. Note the exhaust plume in the center of the photo.

admissions for respiratory conditions when the mill was closed compared to when the mill was operating. NHEERL scientists, in collaboration with academic researchers, obtained particulate matter (PM₁₀) samples from a Utah Valley air monitoring station for the year before (year 1), the year during (year 2), and the year after (year 3) the steel mill closure. Year 2 dust had the lowest concentrations of soluble iron, copper, and zinc, and generated the lowest number of oxidants (reactive compounds believed to be a major cause of lung

tissue damage). Human volunteers were exposed to particulate matter from each of the three years. Dust from years 1 and 3 caused significant lung injury and inflammation, whereas dust from year 2 caused minimal injury. Like the Baltimore and CAPS studies, this study is among the first to demonstrate a correlation between epidemiologic and toxicologic particulate matter research.

This study, and additional laboratory research, suggests a potential pathophysiological mechanism for the health effects reported in the Utah Valley. NHEERL investigators demonstrated that cultured human lung cells exposed to particulate matter from years 1 and 3 produced significantly higher levels of inflammatory mediators than cells exposed to particulate matter



An NHEERL scientist monitors heart rate and blood pressure during inhalation exposure of rats with genetic systemic hypertension to fine ambient air particles from Ottawa, Canada.



Two NHEERL scientists use morphometric analysis and single-cell calcium imaging to demonstrate how particulate matter affects the sensory nervous system in initiating airway inflammation.

from year 2. Researchers are currently using this *in vitro* system to investigate whether transition metals in Utah Valley particulate matter cause the inflammation.

In addition to clinical and laboratory studies in humans, NHEERL scientists used several rodent models of human diseases to study the mechanisms by which particulate matter causes adverse health effects. Rats with hereditary systemic hypertension and cardiac disease serve as a model for humans with high blood pressure and cardiovascular disorders. In one study, these rats were exposed to residual oil fly ash, a type of particulate matter that has a high metal content and is emitted from power plants. Compared

to normal rats, rats with systemic hypertension exhibited exacerbated pulmonary injury, a reduced antioxidant defense response, and adverse electrocardiograph changes. Other models used by NHEERL scientists to study the health effects of particulate matter include a mouse model of allergic airway disease, a rat model of chronic bronchitis, and a rat model of pulmonary hypertension. In an exciting new area of research, NHEERL scientists are using rodent models to study the neurologic mechanisms involved in particulate matter-induced lung inflammation.

¹ Several issues related to particulate matter standards are undergoing Supreme Court judicial review, including the PM_{10} standard. This standard will likely be revised to be $PM_{2.5-10}$, particles smaller than $10\ \mu m$ but larger than $2.5\ \mu m$ in diameter.



The Clean Air Act Amendments (CAAA) of 1990 mandate regulations for air toxics. EPA's air toxics research program provides information that quantifies air toxics health risks and thereby directly supports establishment of these regulations. The air toxics research program investigates and assesses risks posed by toxic air pollutants from major stationary sources such as industries, urban area sources such as dry cleaners, mobile sources including passenger automobiles, and indoor sources associated with houses and buildings. The objective of EPA's air toxics program is to significantly reduce the risk of cancer and other serious adverse health effects caused by air toxics through continuous reductions in emissions. Two major areas of research by NHEERL scientists are (1) carcinogenic mechanisms of air toxics and (2) concentration and time exposure dynamics in the development of risk assessment models.

Clean Air air toxics

CARCINOGENICITY: POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants found worldwide as constituents of complex mixtures in air, water, waste sites, and food. PAHs are specifically listed as Hazardous Air Pollutants (HAP) in the Clean Air Act Amendments of 1990, and are regulated contaminants under the Safe Drinking Water Act and the Clean Water Act. Sources of PAHs include tobacco smoke and emissions from diesel vehicles and industrial processes such as aluminum production, coal gasification, coke production, and iron and steel

founding. Many, but not all, PAHs are known rodent carcinogens and probable human carcinogens.

Complex environmental mixtures containing PAHs have been classified as respiratory carcinogens in humans.

In discovering new metabolic pathways leading to the development of cancer, NHEERL scientists use various analytical techniques, including gas chromatography, mass spectrometry, and nuclear magnetic resonance imaging. Here, a scientist injects a sample for gas chromatography and mass spectrometry analysis.



Because of their structural diversity and wide range of carcinogenic potencies, PAHs are an ideal class of chemicals for studying the relationships between structure and potency, metabolism, biotransformation, biomarkers of dose, biomarkers of effect, and biological activity. In previous studies, NHEERL scientists discovered that individual environmental PAHs varied in their potential to cause lung tumors by a factor of over 200, a critical finding for PAH risk assessment.

In Fiscal Years 1999 and 2000, NHEERL researchers studied the structural features of PAHs that confer carcinogenicity by examining the mechanism by which dibenzo[a,l]pyrene (DB[a,l]P) induces cancer. Dibenzo[a,l]pyrene, the most carcinogenic PAH yet discovered, possesses carcinogenic activity that far exceeds that of benzo[a]pyrene, the archetypal PAH. (See graph.)

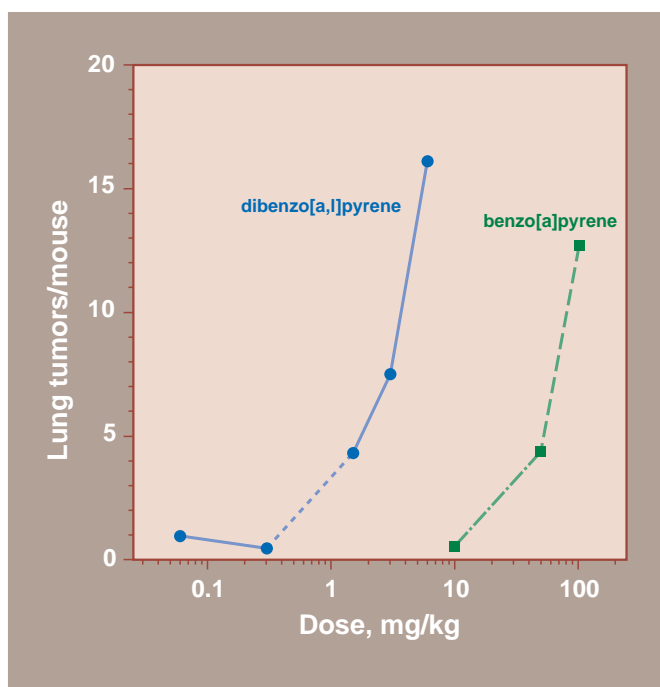
A comprehensive project evaluated the mutagenic and lung carcinogenic effects of DB[a,l]P in bacteria, mammalian cells, and mammals. These studies were complemented with mode-of-action investigations using computational research on DB[a,l]P and structurally related PAHs, metabolic studies, molecular and structural analyses of DB[a,l]P-DNA adducts, and mutagenicity studies identifying the specific mutations induced by these DNA adducts. The results of these studies indicated that several features of DB[a,l]P were associated with its high potency in producing tumors:

- possession of nonplanar structure
- formation of reactive metabolites that avidly bind to DNA
- predilection for binding to adenine residues in DNA
- induction of mutations at adenine:thymine base pairs

In future studies, NHEERL scientists will explore in more depth the relationship between chemical structure and toxic activity.

CONCENTRATION AND TIME EXPOSURE DYNAMICS

In many cases, risk assessment decisions are based on experimental data obtained when animals are exposed to a test substance for one duration of time. However, EPA must assess the risk of exposure for people who



Dibenzo[a,l]pyrene is more potent than benzo[a]pyrene in inducing Strain A/J mouse lung tumors.

are exposed to a substance for varying time intervals. Therefore, risk assessors need strategies for determining how the health risks associated with exposure change as duration of exposure changes. Current risk assessment duration adjustments are based on Haber's rule or a derivation of Haber's rule.

**The simplest form of
Haber's rule is
 $C \times t = K$
where **C** represents the
concentration, **t** the time
(duration) of exposure, and
K a constant toxic effect.**

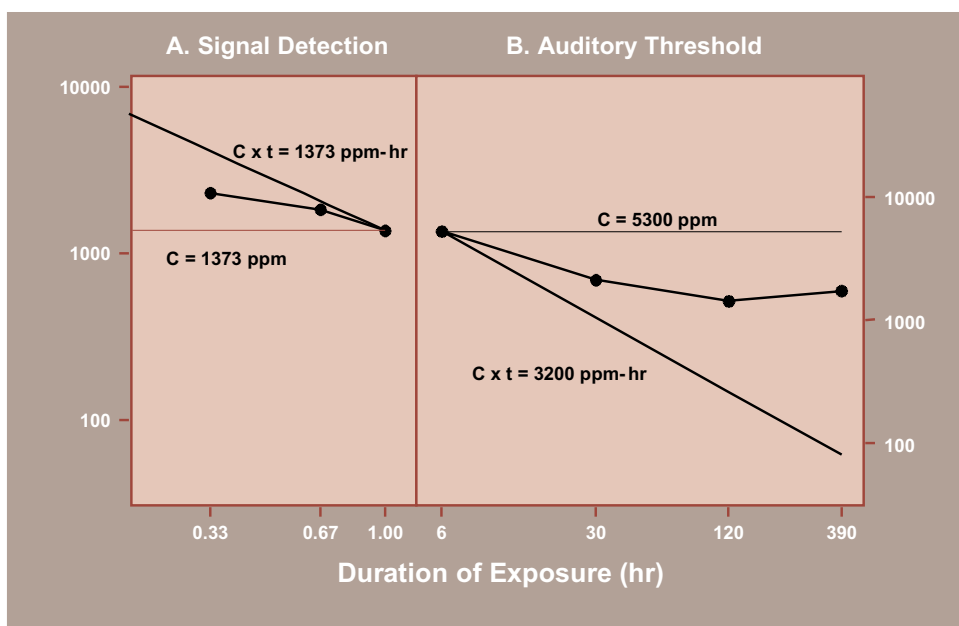
NHEERL scientists used a rodent inhalation study to evaluate the current practice and to examine how estimating tissue dose during exposure might improve accuracy in the risk assessment process.

Trichloroethylene (TCE) is a neurotoxic volatile organic compound. In this study, TCE neurotoxicity was measured by evaluating hearing loss, visual function, and behavior. Tissue dose of TCE at the time of neurological testing was estimated using a physiologically based pharmacokinetic model, which describes the

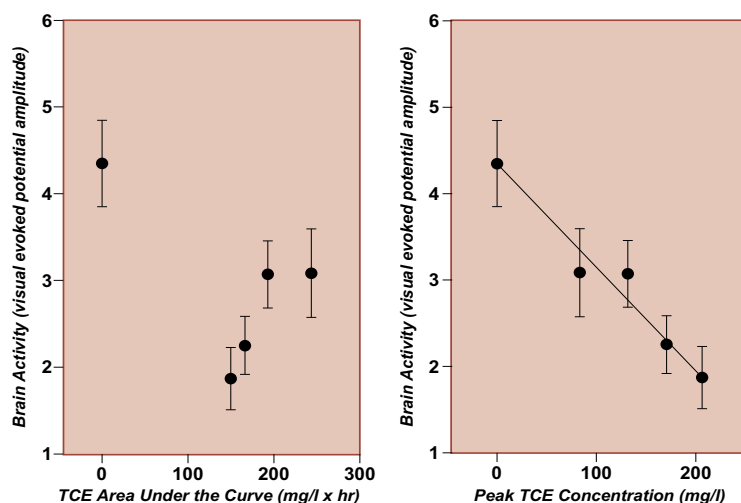
distribution of a substance to specific target tissues over time. The scientists found that, if the simplest form of Haber's rule was used to predict neurotoxic outcomes across different exposure durations, the risk would be overestimated when extrapolating from short to long exposures and underestimated when extrapolating from long to short exposures. For acute effects of TCE on behavior and visual function, the peak TCE concentration in the blood at the time of neurologic testing was a good predictor of observed

performance on neurologic tests. However, cumulative exposure, measured as the area under the blood TCE concentration curve, did not correlate well with outcomes. This study demonstrates that models incorporating tissue dose estimates for varying exposure scenarios will provide more accurate risk assessments than models considering only the external exposure parameters of concentration and time. Similar results have been found in analogous studies that examined changes in tissue sensitivity over time

in the lungs, female reproductive system, and developing fetus.



Signal detection behavior and auditory threshold, actual (•—•) versus predicted (—) by Haber's rule, $C \times t$.



Visual evoked potential amplitude (VEPA) is a measure of brain activity in response to visual stimuli. Peak TCE concentration is a more accurate predictor of VEPA than the area under the cumulative TCE exposure curve.

New Technology Developed

One challenge of studying the neurotoxic effects of inhaled substances results from their rapid clearing from the lung, accompanied by a rapid resolution of symptoms. To study the effects of volatile organic compounds *during exposure*, NHEERL scientists designed and constructed special exposure chambers for rodents that incorporated behavioral and neurophysiological testing facilities with inhalation chambers.

This equipment is used to test rodent visual neurophysiological responses during inhalation exposure to volatile organic compounds. In experiments conducted with this unique apparatus, momentary blood and brain concentrations of the test substances correlated closely with changes in brain function.



This unique experimental system allows NHEERL scientists to assess rodent behavior during inhalation exposure to volatile organic compounds. Experiments conducted in this laboratory demonstrated that a traditional approach to risk assessment based on Haber's rule may misrepresent the risks of behavioral deficits associated with exposures that vary in concentration and duration.



New Genotoxic Mechanism Discovered

NHEERL scientists discovered a new mode of action of PAHs, the K-region activation pathway. The K-region, a structural feature of most PAHs, is activated by mammalian metabolic enzymes, forming K-region dihydrodiols. The researchers showed that the K-region dihydrodiols of several carcinogenic PAHs had intrinsic genotoxic activity. For the carcinogenic environmental contaminants benzo[a]pyrene and dibenzo[a,h]pyrene, this genotoxic activity was apparently not associated with the formation of observable, stable, covalent DNA adducts. This discovery was surprising since the formation of stable, covalent DNA adducts is a common mechanism of genotoxicity and consequent carcinogenicity. These findings suggest that a new class of DNA adduct, hitherto unknown, may be responsible for the genotoxic activity of these metabolic intermediates of PAHs. This newly discovered mechanism of PAH genotoxicity has dramatic implications for the risk assessment process. Because PAHs are released from a wide variety of sources, including tobacco smoke, diesel exhaust, and many industrial emissions, assessing health risks from PAHs is an important area of research.

DRINKING
WATER



The Safe Drinking Water Act Amendments of 1996 mandate that EPA provide a stronger scientific basis to support future regulatory decisions and that EPA conduct research on several specific drinking water contaminants. Current drinking water research priorities include disinfection by-products, waterborne pathogens, and arsenic. There is also a growing emphasis on unregulated chemicals and microorganisms listed on the EPA's Contaminant Candidate List. NHEERL research in two of these areas, waterborne pathogens and naturally occurring arsenic, is highlighted below.

Community water treatment facilities use a variety of techniques to remove or destroy microbial pathogens. These techniques may include filtration and disinfection with either chlorine or an alternative disinfectant such as ozone. EPA's Surface Water Treatment Rule of 1989 requires all communities that use surface water as a drinking water source to filter their water unless special criteria are met. The addition of filtration to municipal water treatment facilities in response to the 1989 rule created unique opportunities for research on waterborne disease occurrence.

Clean Water drinking water

WATERBORNE PATHOGENS

The nature and magnitude of endemic waterborne diseases are not well characterized in the United States. Because these illnesses tend to be self-limiting, causing relatively minor symptoms in most individuals, they are rarely seen by the medical community. However, they may pose a serious health threat to certain groups, particularly young children, the elderly, and the immune compromised. The more common waterborne pathogens are *Shigella* (and other bacteria), *Cryptosporidium*, *Giardia*, and enteric viruses.



Giardia is a common waterborne pathogen.

Based on a survey of water utilities affected by the Surface Water Treatment Rule, NHEERL scientists selected a community for a pilot intervention study. Information on gastrointestinal symptoms was collected before and after installation of a filtration system at the community water treatment plant. Because gastrointestinal illness is more common in children than adults, only families with one or more children were included in the study. Preliminary results showed a significant reduction in the rate of gastrointestinal illness after filtration was instituted. The risk of gastrointestinal illness was 1.8 times greater before filtration than after filtration. One-third (34%) of the gastrointestinal illness that occurred before filtration was attributed to the lack of filtration. Additional analyses are evaluating other

changes in community health that followed addition of filtration to the water treatment process. A larger, similar study was launched in Fiscal Year 2000 in the Seattle-Spokane area and an additional study is planned for a community in Texas.

ARSENIC

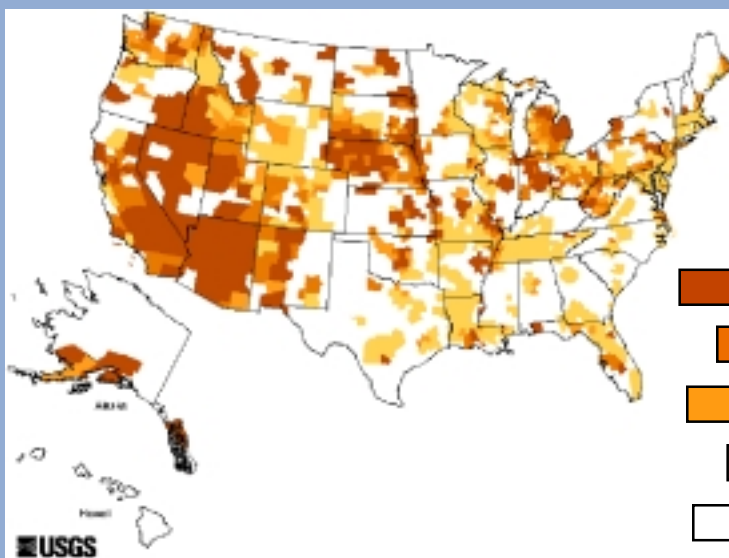
Arsenic occurs naturally in water and soil in certain regions of the United States, particularly in those areas with volcanic or geothermal activity. Prior research has linked the ingestion of arsenic with cancer of the skin and internal organs. Also, evidence suggests that arsenic exposure may be associated with noncancer effects including cardiovascular disease and diabetes. Based on research conducted by NHEERL and other scientists, EPA recently proposed a new Maximum Contaminant Level (MCL) of 5 µg/liter for arsenic in drinking water. The MCL for arsenic has been 50 µg/liter, a standard that was established in 1942.



A water filtration plant under construction in Seattle, WA.

NHEERL scientists conducted an epidemiologic study in Millard County, Utah. The study population was assembled from historic records of the Church of Latter Day Saints (Mormons). This population was chosen due to the expectation that Mormons adhere to lifestyle practices—including abstention from drinking alcohol and smoking tobacco products—that reduce the likelihood that these potentially

Counties with arsenic concentrations exceeding possible new MCLs in 10 percent or more of ground-water samples.



- Counties with arsenic concentrations exceeding 10 µg/L in 10 percent or more of samples.
- Counties with arsenic concentrations exceeding 5 µg/L in 10 percent or more of samples.
- Counties with arsenic concentrations exceeding 3 µg/L in 10 percent or more of samples.
- Counties with fewer than 10 percent of samples exceeding 3 µg/L, representing areas of lowest concentration.
- Counties with insufficient data in the USGS data base to make estimates.

Citation:
Welch, A.H., Watkins, S.A., Helsel, D.R., and Focazio, M.F., 2000, Arsenic in ground-water resources of the United States: U.S. Geological Survey Fact Sheet 063-00, 4p.

confounding factors might have influenced the observed results. All information was obtained from existing records, including death certificates. Arsenic exposure was not measured directly, but was estimated based on historic measurements of arsenic in the drinking water. (In the wells of this region, inorganic arsenic levels vary from 2 $\mu\text{g}/\text{liter}$ to more than 600 $\mu\text{g}/\text{liter}$.) Since mortality records were examined for cause of death, scientists did not obtain information on other sources of arsenic exposure. Therefore, although the results of this study suggest an association between arsenic exposure and adverse health effects, they do not firmly establish a cause-and-effect relationship. Obtaining data only from existing records imposes limitations on study design and interpretation of results, but it is a time-efficient and cost-effective way to identify issues worthy of further investigation.

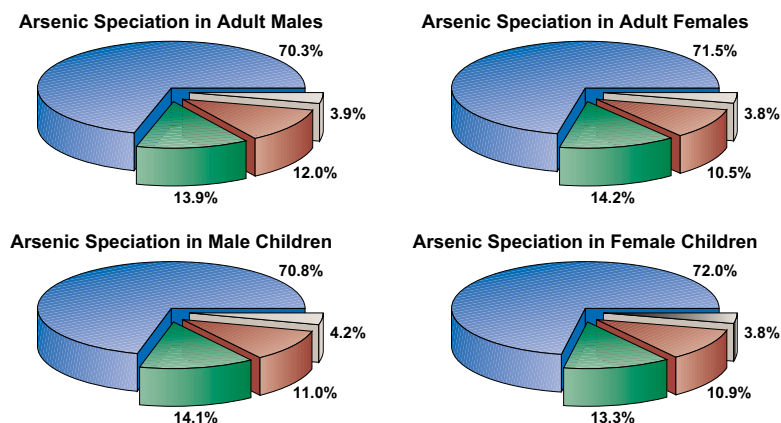
Mortality in the study population was compared with that expected in the Utah general population. Among men in the study population, prostate cancer, hypertensive heart disease, and one type of kidney disease occurred at higher rates than expected. Among women in the study population, hypertensive heart disease and other types of heart disease occurred more frequently than expected. No other health outcomes in the study occurred at an increased rate.

Other NHEERL scientists are exploring the potential health effects of arsenic in drinking water in more detail. One study examined

An NHEERL scientist collects a water sample from the kitchen sink of a household participating in an epidemiologic study.

urinary arsenic level as a potential biomarker of exposure in a chronically exposed population. Arsenic concentrations in the drinking water varied from 8 to 620 micrograms per liter. Arsenic intake was estimated using two data sources: daily food diaries kept by participants and inorganic arsenic levels in the drinking water. In the body, inorganic arsenic is metabolized into several forms. Researchers found that all age and gender groups in the study excreted the various forms of arsenic in the same proportions. (See pie charts.)

Arsenic Profiles for Different Groups

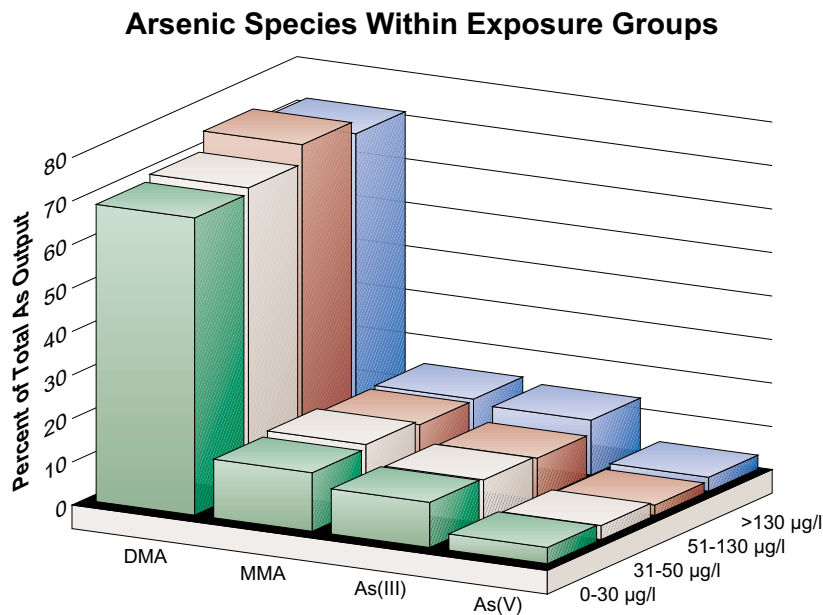


Regardless of age or gender, the same arsenic compounds (species) were produced in similar proportions.

This proportion also remained constant over all levels of arsenic exposure. (See bar graph.)

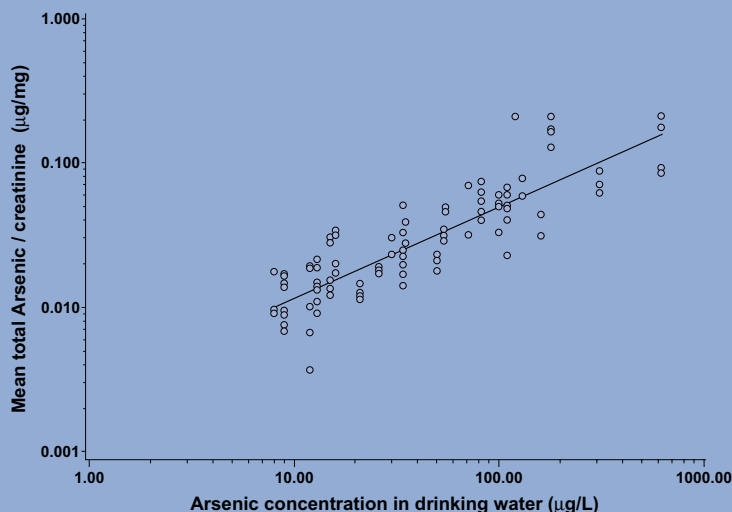
The study also showed that the concentration of arsenic in drinking water is a better predictor of urinary arsenic excretion than estimates of arsenic

consumption calculated from daily food diaries. (See line graph.) Therefore, urinary arsenic concentration can be used to accurately estimate exposure in future studies of the potential health effects of arsenic in drinking water.



In all exposure levels, the same arsenic compounds (species) were produced in similar proportions.

The concentration of arsenic in drinking water is a better predictor of total urinary arsenic excretion than estimates of water consumption from food diaries. (Urinary arsenic level was divided by urinary creatinine in order to adjust for total urine solute concentration.)



As a complement to epidemiologic and clinical studies, and to provide additional scientific data for the risk assessment process, NHEERL scientists working in the laboratory study the tissue distribution and metabolic fate of arsenic in rodents.

An NHEERL researcher loads tissue samples from animals treated with radioactive arsenic into a liquid scintillation counter. This process enables scientists to determine the organs and tissues in which arsenic accumulates in the body.



NHEERL scientists characterize the arsenic metabolites in the urine of exposed animals using high performance liquid chromatography coupled with flow scintillation analysis.



Various types of chemical, biological, and physical stressors affect the health and sustainability of fragile aquatic ecosystems. To improve ecosystem risk assessment, EPA conducts research to identify and assess the impact of aquatic stressors. Three critical types of stressors are being studied by NHEERL scientists: chemical stressors, such as persistent bioaccumulative toxicants; nonchemical stressors such as habitat alteration; and eutrophication, which includes the effects of nutrient overload, hypoxia, and harmful algal blooms.

providing a better understanding of risks to ecosystem resources and processes, this research will promote better and more ecologically sustainable choices by a variety of decision makers.

NHEERL researchers obtaining samples of aquatic animals from a stream.



NHEERL's Wildlife Research Strategy addresses four key areas of research where advances in science will dramatically improve wildlife risk assessment techniques and criteria methodology:

Clean Water aquatic stressors

WILDLIFE RESEARCH STRATEGY

Over the past year, EPA's NHEERL finalized the Wildlife Research Strategy, which describes the strategic approach for NHEERL scientists to follow when researching the effects of environmental stressors on wildlife. The goal of the Wildlife Research Strategy is to develop scientifically valid approaches for assessing risks to wildlife populations from multiple stressors.

Research conducted under this program will produce models, methods, and findings for EPA Program and Regional Offices to use when conducting wildlife population risk assessments. By



- Extrapolation research will improve the basis for predicting toxicological responses among wildlife species and exposure scenarios of concern.
- Coordinated wildlife population biology and wildlife toxicology research will improve predictions of population dynamics in spatially explicit habitats.
- Methods research will advance techniques for assessing the relative risk of chemical and nonchemical stressors on wildlife populations. The program uses a tiered approach to developing tools, recognizing that different tools are needed for screening-level assessments and for scenario-specific risk assessments.
- Additional research will define geographical regions and spatial scales appropriate for wildlife risk assessments.

An NHEERL scientist is developing statistical and ecological models using the estuarine benthic amphipod *Ampelisca abdita*, a bottom-dwelling crustacean, to evaluate sediment toxicity. These models describe the relationship between classical toxicological endpoints and population-level effects measured in field monitoring programs.

During Fiscal Year 2000, the Wildlife Research Strategy underwent external review and was finalized. In addition, the NHEERL's Wildlife Workgroup

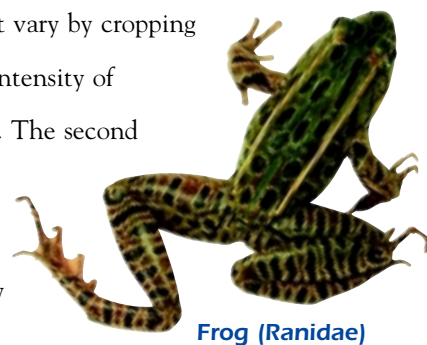


Green Heron (*Butorides striatus*)



**Bald Eagle
(*Haliaeetus leucocephalus*)**

identified three case studies that will be used to demonstrate the utility of models and methods developed using this strategy. These three risk assessment scenarios represent a broad diversity of environmental issues. The first scenario will examine the impact of acutely toxic agricultural insecticides on representative bird species. This project will focus on population-level effects in agricultural landscapes that vary by cropping practices and intensity of insecticide use. The second scenario will examine the role of mercury and other coexisting stressors on population dynamics of common loons and other fish-eating birds. The third scenario will examine the suite of stressors that affect amphibian population dynamics.

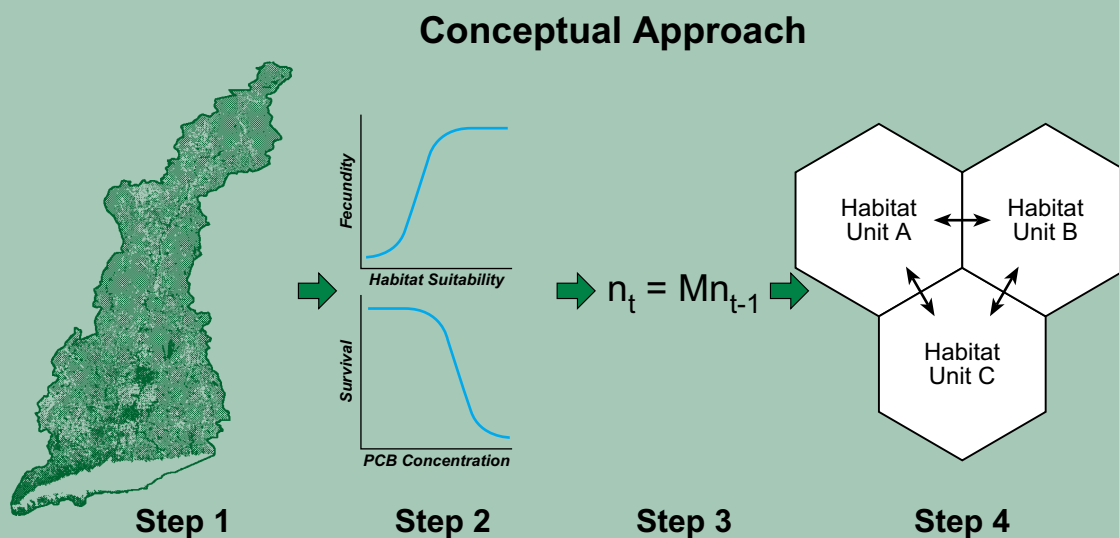


Frog (*Ranidae*)

The Wildlife Research Strategy is an excellent example of how EPA scientists develop the methods and procedures by which research is to be conducted. Because the problems addressed by this program are multidimensional—requiring the integration of researchers from such diverse fields as toxicology, population ecology, and spatial modeling—this program is also an outstanding example of the integrated approach that EPA uses to address complex environmental issues.

WILDLIFE RESEARCH STRATEGY

The figure below outlines the NHEERL conceptual approach for wildlife risk assessments, focusing on the effects component of the assessment process. **Step 1** involves spatial and temporal characterization of stressors that may adversely affect the population of concern, especially contaminant exposure, habitat suitability, and introduced species. Results from Step 1 provide the input for **Step 2**, quantification of the exposure-response and habitat-response relationships at the individual level. The specific response variables estimated in Step 2 are spatially explicit demographic rates of individuals within populations, such as fecundity and life stage-specific probability of survival. These demographic rates in turn drive population models in **Step 3**, generating outputs describing population growth rate or other appropriate population-level endpoints (for example, extinction probabilities). The formula in Step 3 is a very simplistic way of saying that the population size at some time period (n_t) is related to the population size at the previous time period (n_{t-1}) times M , the population growth rate, which integrates survival, fecundity, immigration, and emigration. Finally, these population dynamics are integrated with the landscape characteristics in **Step 4** to determine habitat-specific population sources and sinks using spatially explicit modeling platforms. Analysis of the cumulative population dynamics across the landscape provides the estimates of wildlife risks from chemical exposure, habitat changes, introduced species, and other forms of disturbance in the landscape.



Conceptual approach to wildlife risk assessment. The four steps are landscape characterization, development of exposure and habitat response relationships, estimating population responses, and spatial modeling. In Step 3, n_t = population size at time t , M = population growth rate, n_{t-1} = population size at the previous time period.

DISSOLVED OXYGEN

Just as oxygen in the air is critical to the life of land animals, oxygen dissolved in water is vital to the survival of aquatic animals. Oxygen enters water from the atmosphere by diffusion and by the action of wind on the water surface. Floating and rooted aquatic plants add dissolved oxygen to shallow waters that receive enough sunlight to support photosynthesis. Under normal conditions, temperature and salinity are the two most important factors that influence the amount of oxygen that can be dissolved in a body of water.

Low levels of dissolved oxygen stress aquatic animals and can cause death if the levels are low enough. Eutrophication — increased nutrients in the water — is the most common cause of low dissolved oxygen levels. Wastewater discharges and agricultural and urban practices that result in nutrient-rich runoff are the activities most responsible for eutrophication of streams, rivers, lakes, and estuaries. Increased nutrient levels promote algal blooms. The subsequent decomposition of dead algae consumes oxygen, lowering the dissolved oxygen content of the water. Massive fish kills in estuaries often make the news headlines during the summer months, when the effects of eutrophication are most severely felt.

Congress has mandated the establishment of national minimal levels for dissolved oxygen. In FY 2000, NHEERL researchers completed a dissolved oxygen criteria document for saltwater, which describes sensitivity to low dissolved oxygen levels in saltwater animal species of the mid-Atlantic region. Most of these species also occur along the southern coasts of

the United States. Because higher water temperatures are expected to increase respiratory demand for dissolved oxygen, and less oxygen is dissolved in warm *versus* cold waters, scientists hypothesized that southern populations may differ in their sensitivity to low dissolved oxygen levels compared to northern populations of the same species.



To test this hypothesis, NHEERL researchers compared sensitivity to dissolved oxygen in northern and southern populations of two crustaceans (Say mud crab, *Dyspanopeus sayi* and grass shrimp, *Palaemonetes vulgaris*) and one fish (inland silverside, *Menidia beryllina*). The scientists selected crustacean populations from Rhode Island and Georgia and fish populations from Rhode Island and Florida. Rhode Island populations were tested at 18 to 20°C, whereas southern populations were tested at 28°C.



Researchers conducted acute exposure tests on juvenile fish and on larvae of all three

Heat-exchange system in seawater trough that controls test temperature in experiments with marine and estuarine organisms.

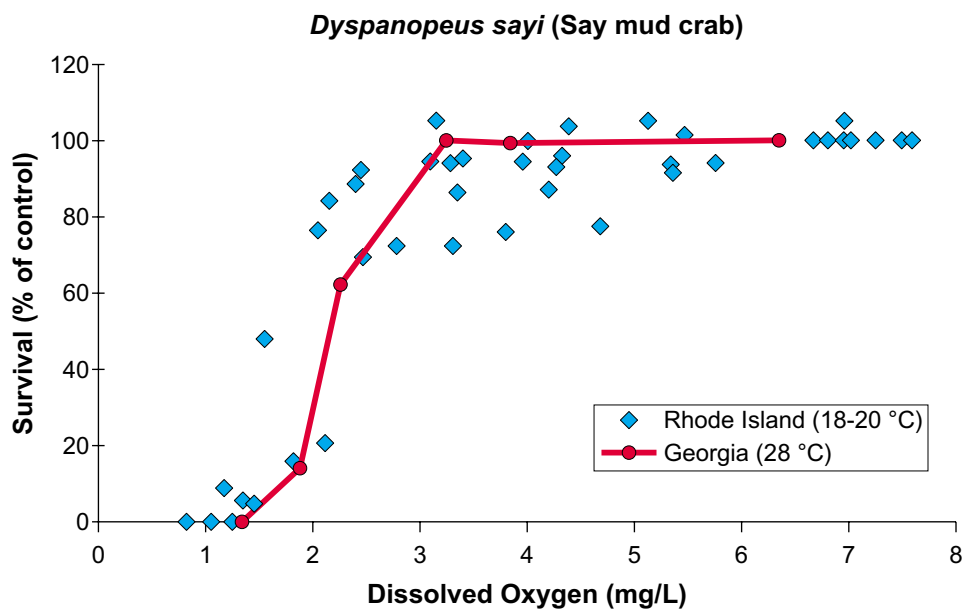
species. All tests were conducted in a flow-through system capable of maintaining dissolved oxygen content at any level between zero and saturation.

In FY 2000, tests on all three species were completed and showed that northeastern and southern populations of these three species displayed the same sensitivity to low dissolved oxygen levels. (See graph.) This research demonstrates that much of the data used in establishing the mid-

Atlantic dissolved oxygen criteria can be used in developing dissolved oxygen criteria for the coastal waters of the southeastern U.S. and Gulf of Mexico. Ongoing research will answer remaining questions

about applying the methods and data of the Mid-Atlantic dissolved oxygen studies to other coastal regions. This is one example of NHEERL research that directly supports the development of regulatory standards.

NHEERL scientist working at the facility used to study the effects of low dissolved oxygen on marine organisms.



Say mud crabs from Georgia exhibited the same sensitivity to dissolved oxygen levels as say mud crabs from Rhode Island.



The Food Quality Protection Act of 1996 (FQPA) mandates development of a single, health-based standard for all pesticides in all foods and provides special protection for sensitive subpopulations, particularly infants and children. The FQPA also directs EPA to consider all nonoccupational sources of exposure, including drinking water, when setting maximum allowable levels for contaminants. In support of these mandates, NHEERL scientists are actively studying age-related differences in response to pesticides, the effects of aggregate and cumulative exposures, qualitative and quantitative differences, and improved methods for extrapolation. This research is being conducted as a collaborative effort among divisions within NHEERL.

response to a single oral dose five- to seven-fold lower than the dose that caused toxic responses in adult rats. The toxic response measured was either a behavioral or biochemical abnormality consistent with anticholinesterase exposure. This response correlated with the age-related development of the enzyme systems responsible for chlorpyrifos detoxification.

“...age-related differences in toxicity occur for some pesticides but not for others.”

Food Quality Protection pesticide residues in food

Researchers at NHEERL have developed a rodent model to study how age influences the response of an individual to a given pesticide dose. Chlorpyrifos [Dursban®, Lorsban®, O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is an anticholinesterase chemical that has been one of the most widely used organophosphate insecticides in the United States. In studies of chlorpyrifos, young rats exhibited a toxic

However, rats exposed to methamidophos (Monitor®, O,S-dimethyl phosphoamidothioate), which is detoxified by different mechanisms, showed no age-related difference in toxic response. This comparison demonstrated clearly that age-related differences in toxicity occur for some pesticides but not for others. Further research is aimed at understanding the underlying basis for these differences, so that conducting studies on a chemical-by-chemical basis may not be necessary.

NHEERL scientists are using a variety of approaches to study the effects of mixtures of pesticides that share a common mechanism of toxicity. The researchers are

- developing statistical models to assess additive and nonadditive interactions.
- conducting interaction experiments of two to five pesticides.

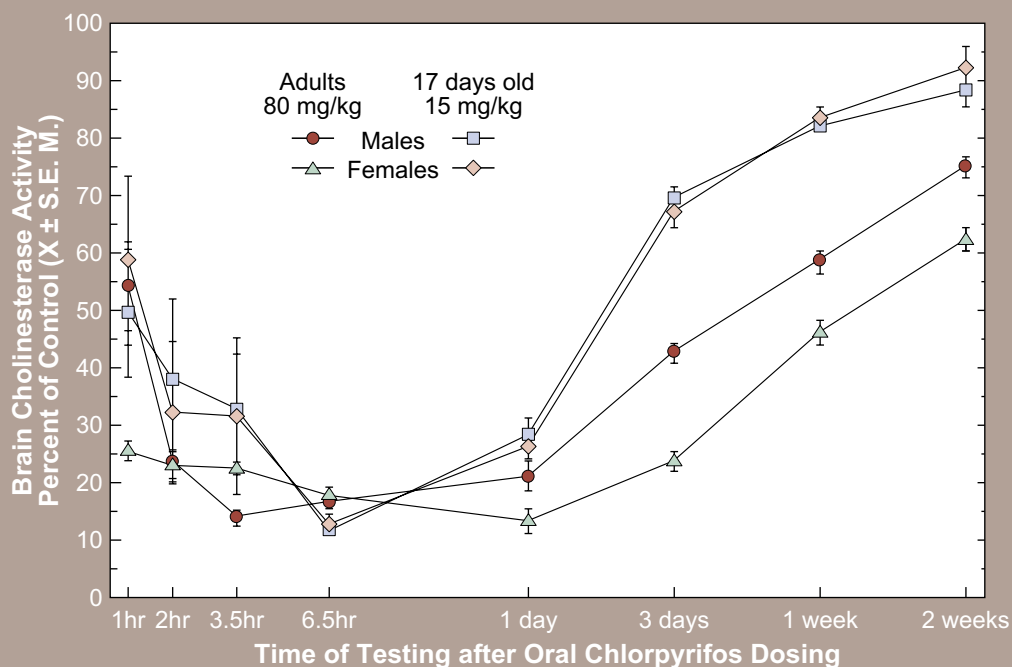
- determining age-related differences in response to pesticide mixtures.
- evaluating effects on behavior, neuromuscular function, central nervous system electrophysiology, thermoregulation, and cardiovascular function.

In one pesticide mixture study, the toxicity of a blend of chlorpyrifos and diazinon showed no deviation from additivity.

Age-related Differences in Response to Chlorpyrifos

Compared to adult rats, young rats

- exhibited a toxic response at a five- to seven-fold lower dose.
- exhibited a peak toxic response more slowly.
- recovered and returned to normal cholinesterase activity more quickly.
- showed no gender-related differences in toxic response.





Changes in Chlorpyrifos Use

In June 2000, EPA released revised risk assessment and risk mitigation measures for chlorpyrifos. Based on this revised risk assessment, the Agency has targeted the end of 2001 to implement major changes in both commercial and homeowner use of this pesticide. NHEERL scientists contributed some of the key research upon which the chlorpyrifos revised risk assessment and risk mitigation measures were based.



People may be most vulnerable to certain classes of environmental contaminants during *in utero* development, infancy, childhood, and/or adolescence.

Children may be more vulnerable to environmental contaminants than adults because of differences in absorption, metabolism, storage, and excretion, the sum of which produces higher biologically effective doses in target tissues. Furthermore, children and adolescents can have greater exposure than adults because of proportionately higher food intake, dietary differences, and activities that result in greater contact with environmental contaminants.

Many environmental health threats to children may not be recognized because scientific understanding of when and why children's exposures and responses differ from those of adults is

PESTICIDES AND THE DEVELOPING IMMUNE SYSTEM

EPA's Office of Prevention, Pesticides, and Toxic Substances recently identified the need to evaluate



immune function in developing animals. In FY 2000, NHEERL scientists validated a rodent model to detect increased immune dysfunction in adults following

Safe Communities pesticides in the environment

incomplete. The Food Quality Protection Act of 1996 and the Safe Drinking Water Act Amendments of 1996 require that EPA give special consideration to children and other susceptible subpopulations when establishing health-based standards, especially those related to pesticides in food and water.

chemical exposure during immune system development. This standardized procedure for evaluating developmental immunotoxicity can be used by other researchers and by the chemical industry to investigate the developmental immunotoxic potential of individual agents or mixtures. In this way, relatively

small research projects completed in the laboratory provide the means to collect millions of dollars worth of data generated by chemical manufacturers.

“...following chemical exposure during immune system development, the fetus, neonate, young child, adolescent, and/or adult may develop chemical-induced immune dysfunction that may manifest as a susceptibility to infectious diseases or the development of cancer.”

In validating this model, NHEERL researchers exposed rats to the organochlorine chemical TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) and to the organochlorine pesticides heptachlor and methoxychlor. Pregnant females exposed to TCDD on gestation day 14, resulting in late gestational and early lactational exposure, gave birth to pups that exhibited persistent suppression of T cell-mediated immune responses. Male offspring tended to be more sensitive to immune suppression than female offspring. In separate studies of perinatal/juvenile exposure to heptachlor and methoxychlor, NHEERL scientists discovered that the amount of major metabolites of these pesticides found in dam's milk and in lymphoid and other tissues was directly proportional to immune system suppression. That is, the greatest amount of

immune system dysfunction occurred in the rats with the highest levels of pesticide metabolites. In contrast, *adult* rats exposed to comparable doses of these three chemicals did not exhibit immune suppression.

T cell-mediated immune functions are important in the defense against bacterial, viral, and parasitic infections, and against certain cancers. These studies suggest that, following chemical exposure during immune system development, the fetus, neonate, young child, adolescent, and/or adult may develop chemical-induced immune dysfunction that may manifest as a susceptibility to infectious diseases or the development of cancer. These studies also underscore the need for further experimental and epidemiological work in developmental immunotoxicology to support EPA efforts in children's risk assessment.

PESTICIDES IN THE COMMUNITY

NHEERL researchers conducted a pilot study of children in southern California's Imperial Valley to evaluate organophosphate pesticide exposure and potential health effects. Organophosphate pesticides are used residually and agriculturally, and are the most commonly used class of insecticides in the United States today. Because agricultural application of pesticides is a primary potential route of human exposure, the Imperial Valley, with its year-round agricultural activities, was an ideal site for this study.

Children two to four years of age with flu-like symptoms who were being seen by a physician at a health clinic were eligible for the study. With the informed consent and assistance of the parent or guardian, scientists obtained a urine sample from each child for organophosphate pesticide metabolite analysis.

Researchers also requested a finger-prick blood sample to analyze for the enzyme acetylcholinesterase, but the blood sample was not required for participation in the study. The two basic goals of the study were to (1) estimate the occurrence of unrecognized pesticide-related illness in young children and (2) improve methods to evaluate the prevalence of pesticide exposure among young



Aerial spraying of pesticides is a common practice in many agricultural regions.

children. By the end of Fiscal Year 2000, data had been collected and were being analyzed. This study will provide important information about pesticide exposure in children that will directly support EPA's regulatory efforts.

Organophosphate pesticide exposure may cause flu-like symptoms without fever. Symptoms may include some or all of the following.

- Headache
- Dizziness
- Abdominal pain
- Blurred vision
- Muscle aches
- Vomiting
- Diarrhea
- Loss of appetite
- Muscle twitching or incoordination
- Constricted pupils
- Excessive sweating or tearing
- Breathing problems



In 1990, Congress established the U.S. Global Change Research Program (USGCRP) to coordinate federally supported research on global climate change. As part of the USGCRP, EPA conducts assessments research on the consequences of global climate change to human health, ecosystems, air quality, and water quality. Stressors of concern include (1) increasing temperature, (2) decreasing stratospheric ozone levels, which increase harmful ultraviolet radiation exposure, and (3) changing land cover and land use, which sometimes have unintended and adverse consequences.

would have a major impact on coastal areas because of the high population density of such regions, substantial costs of defending shorelines and property, detrimental effects on infrastructure (drinking water supplies, waste management systems), and significant loss of coastal wetlands, beaches, and recreational facilities.

Global climate change researchers at NHEERL are developing assessment tools to:

- document historical variations in environmental conditions in estuaries and watersheds over the past few centuries.
- associate these variations with changes in stressors.
- differentiate effects in estuaries that result from natural *versus* man-made causes.

Climate Change global climate change

COASTAL AREAS

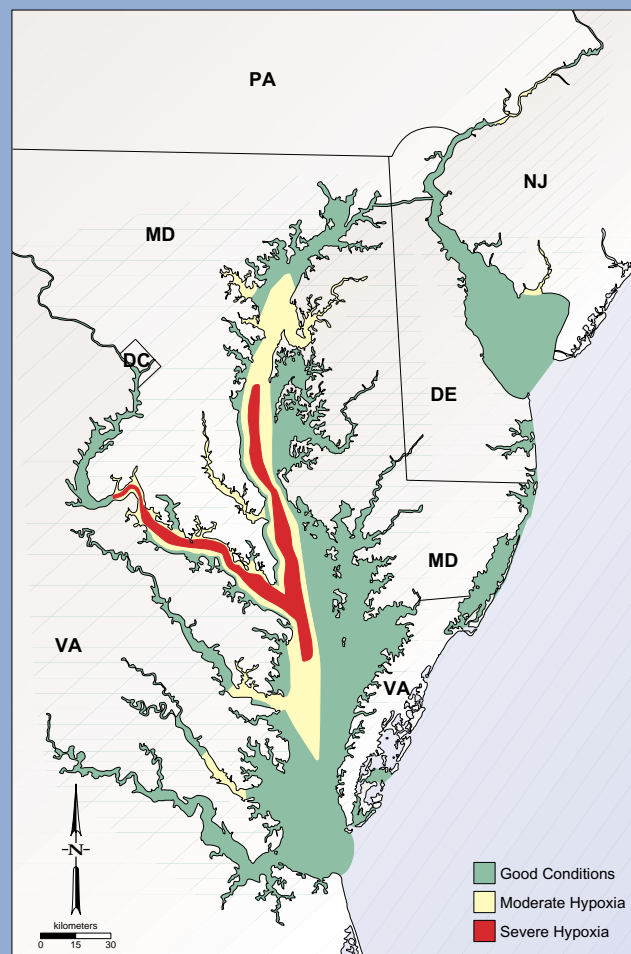
Continued increases in concentrations of atmospheric greenhouse gases can raise global surface temperature—accelerating the hydrological cycle, changing the patterns of climate variation, raising the sea level, and possibly altering the frequencies and intensities of climate extremes. Risks to human health and ecosystems may occur either as the direct result of such climate changes (for example, heat-related mortality or sea-level rise) or from the combined effects of climate variation and other stressors affecting air and water quality. A rising sea level

At NHEERL, global change research is supporting EPA's ability to perform effects-based assessments that integrate across ecological resources and geographic scales and that address major classes of environmental problems. NHEERL scientists are developing methods and collecting data to characterize current ecosystem conditions in mid-Atlantic and Northeast estuaries. Their objective is to relate ecological conditions in these estuaries to past and current characteristics—topography, vegetation type and coverage, and human land use—of the associated watersheds.

Projected climate extremes in the 21st century, such as floods and droughts, may be of greater magnitude than those experienced in the 20th century. Recent results from NHEERL studies suggest that human population trends and resulting modification of watersheds in the mid-Atlantic region over the past fifty years have amplified some risks associated with climate extremes. Increased demands for water can magnify effects of drought. Increased nutrient loading to watersheds has substantially raised the nitrogen flux-per-unit flow over the past century. Preliminary results suggest that, in recent decades, hypoxic and anoxic conditions in the Chesapeake Bay have become more sensitive to climate variation, possibly due to increased nitrogen loading to associated watersheds. Similar effects have been observed in the Gulf of Mexico. Since the late 1950s, annual delivery of nitrate from the Mississippi River to the Gulf has nearly tripled, resulting in an expanding hypoxic zone on the Louisiana-Texas shelf. The findings of research conducted by NHEERL and other USGCRP scientists suggest that risk managers should consider adaptive actions to minimize health and ecological risks associated with both direct and indirect effects of climate variability and change.

FORESTS

NHEERL scientists have analyzed the effect of interactions of climate change, elevated carbon dioxide, and ground-level ozone on biomass accumulation. Modeling studies indicated that the amount of carbon that can be stored as biomass under increasing carbon dioxide levels is reduced with increasing ground-level ozone exposure. A study of Douglas fir seedlings showed that elevated



Distribution of summertime dissolved oxygen within one meter of bottom sediments across estuarine waters in the Mid-Atlantic Region. Data were derived from daylight observations and do not necessarily reflect nighttime depressions that may occur in some areas. (Condition of the Mid-Atlantic Estuaries EPA 600-R-98-147).

atmospheric carbon dioxide concentrations reduced the levels of monoterpenes released by the seedlings. Similarly, elevated temperatures also reduced monoterpene release significantly. These findings have major implications for Douglas fir forests because monoterpene emissions are an important ecological defense against leaf predation by insects.

ULTRAVIOLET RADIATION

Whereas high concentrations of *ground-level* ozone pose risks to human and ecosystem health, high concentrations of *stratospheric* ozone protect human

and ecosystem health by absorbing harmful ultraviolet (UV) radiation. Stratospheric ozone is being depleted by chemical emissions, especially chlorofluorocarbon compounds that have been widely used as refrigerants and aerosol propellants. Exposure to ultraviolet radiation has been associated with immune system suppression, cataract development, and increased risk of some skin cancers.

The EPA program UV-Net monitors ultraviolet radiation at the Earth's surface. Data are collected



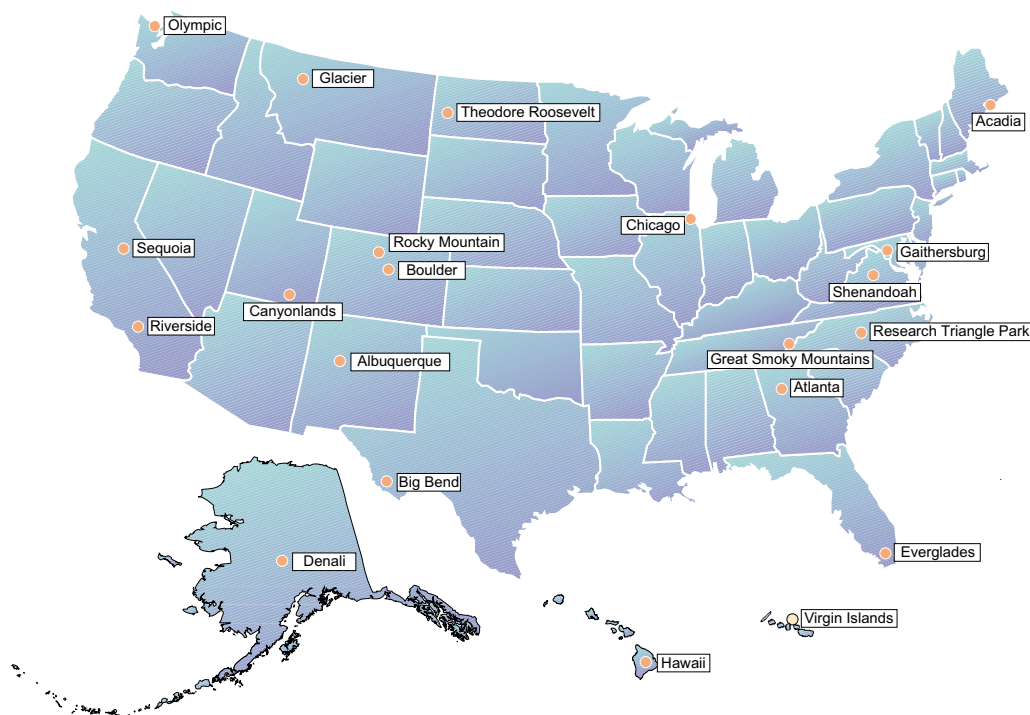
from monitoring sites in 7 urban areas and 14 national parks, which represent major

An ultraviolet (UV) radiation monitor.

ecosystem types. Park service personnel operate the monitoring sites in the national parks, which also collect air quality data. The UV radiation data from this program is coordinated with data collected by other agencies and made available to researchers through the National UV Monitoring Center (NUVMC). The NUVMC is a cooperative endeavor of the EPA and the University of Georgia.

Other NHEERL researchers have studied the influence of ultraviolet radiation on the toxicity of polycyclic aromatic hydrocarbons (PAHs). PAHs are ubiquitous pollutants that are found in tobacco smoke and emissions from diesel vehicles, coal gasification plants, and many industries. Scientists exposed the segmented worm *Lumbriculus variegatus* to phototoxic PAHs (anthracene, fluoranthene, and pyrene) singly and in binary mixtures. Afterward, the worms were exposed to ultraviolet light. The resulting toxicity

Locations of UV monitoring stations



was described by a concentration addition model. That is, exposure to PAHs and ultraviolet light was more toxic than exposure to PAHs alone. This research is just one example of how NHEERL scientists are thinking about the potential interaction of environmental stressors that are traditionally studied by researchers in widely separated disciplines.

NHEERL ecologists are studying the decline of corals in recent decades, especially in the Caribbean Sea. Coral reefs have a very high biodiversity and abundance of life. Coral losses have been attributed to disease and coral bleaching — the loss of obligate symbiotic algae from the coral tissue. Several causes of bleaching and disease have been proposed, including increased exposure to ultraviolet radiation, increased

water temperatures, higher nutrient concentrations, and combinations of these stressors. Coral ecosystems are sufficiently important that an Executive Order was issued to improve understanding of the factors causing coral loss, with the goal of curtailing further loss.

A Florida Keys field survey completed in FY 2000 determined frequency and distribution of coral diseases and bleaching. The survey included permanent sites that are revisited periodically to monitor long-term trends. Data from additional survey sites will allow comparison of different regions and reef types. Data from all survey sites will be examined for potential relationships with UV exposure, temperature, and water quality.



NHEERL scientist examining specimens exposed to UV radiation in a solar simulator.

NHEERL scientists also participate in the Coastal Intensive Site Network (CISNet). As part of CISNet, researchers are conducting field transplant and laboratory studies of corals and symbiotic algae. In these studies, coral measurement endpoints include bleaching, symbiotic algae counts, tissue protein content, thymine dimer production (related to UV exposure), pigment formation (potential protection from UV exposure), and severity of diseases.



NHEERL's Coral Research Program: FY 2000 Accomplishments

- Developed and implemented sampling methods and a probabilistic sampling design for measuring the distribution and frequency of coral disease in the Florida Keys.
- Completed a stratified random survey to compare coral disease prevalence across regions and reef types in the Florida Keys.
- Measured coral bleaching endpoints on samples collected throughout the diurnal cycle and transplanted at different depths, corresponding to different UV exposures. Measured coral bleaching endpoints on laboratory samples of five coral species (three from Florida and two from Hawaii) exposed to various doses of full solar irradiance (standardized to field readings) with and without UV.
- Exposed laboratory corals to UV radiation, demonstrating an inverse relationship between protective coral pigments and thymine dimer formation (an indicator of UV-induced DNA damage).
- Designed and constructed a paired-tank laboratory system for culturing and maintaining experimental corals and testing simple variables. Constructed a UV-simulator system for laboratory exposures.



Snowy Egret (*Egretta thula*)

In recognition that knowledge is vital to policy-making decisions, EPA conducts ecosystems research to provide the scientific understanding needed to maintain or restore the integrity of ecosystems now and in the future. This research provides information at multiple geographic levels: local, watershed, state, regional, tribal, national, and international.



NHEERL ecologists working in a coastal mud flat. Hovercraft used to gain access to the research site is shown in the background.

Scientists at NHEERL are leaders in ecosystem research. The Agency's ecosystem research program consists of four fundamental areas: monitoring,

ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM

The Environmental Monitoring and Assessment Program (EMAP) was created to establish a scientific basis for statistically valid, cost-effective assessments of the condition of various ecosystems and to detect trends at local, state, regional, and national levels. Since its inception in 1988, scientists in the EMAP have

- developed ecosystem-specific indicators and monitoring designs.
- determined baselines for the health of some aquatic ecosystems.
- developed technologies and procedures that reduce monitoring costs.
- identified areas for restoration and remediation.
- transferred technology to some states for collecting monitoring data, which could be aggregated for national assessments.

"Proof-of-concept" studies, which validated the EMAP's approach to ecosystem monitoring, were conducted in the mid-Atlantic region and were completed in 1999. Two new programs, the Coastal 2000 Initiative and the Western Pilot, were designed

Sound Science

ecosystems protection

processes and modeling, risk assessment, and risk management and restoration. Current NHEERL research projects are establishing the baseline health of ecosystems throughout the United States and are developing new, efficient procedures for ecosystem monitoring and assessment.



Measuring light-harvesting efficiency of salt-marsh plants.



For the Coastal 2000 Initiative, coastal areas of the United States have been assigned to named provinces.

to assess the health of aquatic ecosystems on a large scale. They were launched in 1999 and continued through 2000.

The Coastal 2000 Initiative will provide a comprehensive, statistically valid estimate of the health of the nation's estuaries at the state, regional, and national levels. It involves a strategic partnership among EPA, other federal agencies, 24 coastal states of the United States, and Puerto Rico. In 1999, NHEERL and state personnel assessed the condition of small estuaries—those less than 250 square kilometers in size—in California, Oregon, and Washington. In 2000, these assessments were extended to the Atlantic and Gulf coasts with over 1,500 sites being sampled. Initial findings are expected in 2001.

The Coastal 2000 Initiative provides the first national environmental report card on coastal ecosystem health.

The Coastal 2000 Initiative will supply the EPA with important baseline data about the health of our nation's estuaries.

The Western Pilot is the largest, most comprehensive study sponsored by the EPA on the ecological condition of the western part of the United States. It is a collaborative effort of 12 western states,

native American tribes, universities, three

EPA Regions, and other federal agencies. The

objectives of this project are to establish the

baseline health of the widely varied aquatic

ecosystems throughout the west and to identify

environmental stressors associated with degraded

conditions in these systems. In Fiscal Year 2000, the

large estuaries of the west coast—Puget Sound, WA;

Columbia River Estuary, OR; and San Francisco Bay,

CA—were studied.

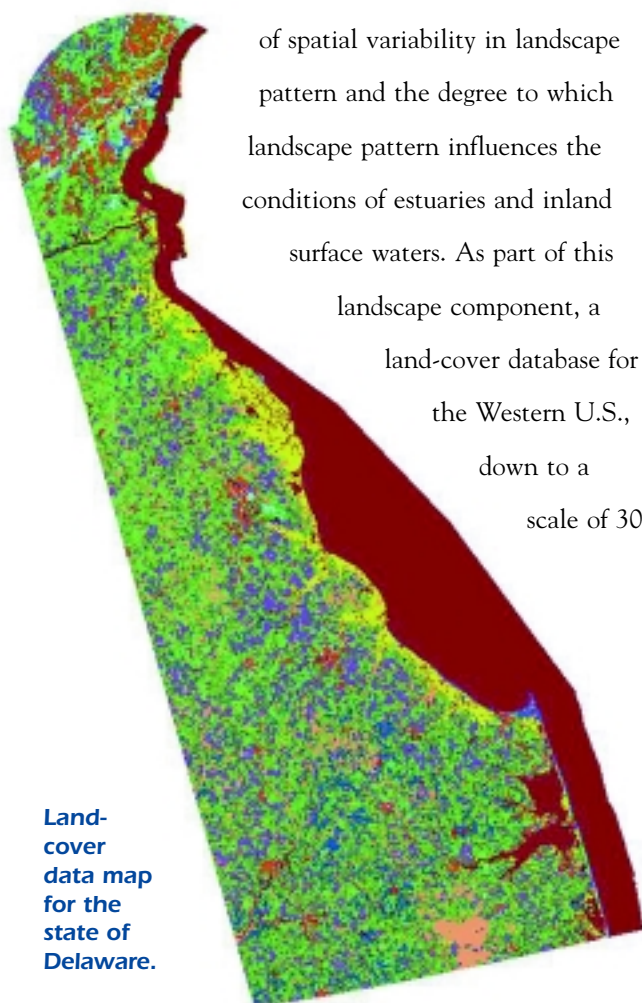


NHEERL scientist collecting samples in a stream.

The Western Pilot is a collaboration with 12 western states, native American tribes, universities, three EPA Regions, and other federal agencies.

The large-scale evaluation of streams and rivers in the Western Pilot is designed to validate (in western aquatic ecosystems) the stream survey design, biological indicators, and estimates of ecological health developed in the earlier Mid-Atlantic Integrated Assessment (MAIA). A third component

of the Western Pilot is the assessment of spatial variability in landscape pattern and the degree to which landscape pattern influences the conditions of estuaries and inland surface waters. As part of this landscape component, a land-cover database for the Western U.S., down to a scale of 30



Land-cover data map for the state of Delaware.

square meters, is being constructed using remote sensing techniques and Multi-Resolution Land Characteristics (MRLC) data.

LAKE SUPERIOR BASIN WATERSHED STUDY

The Lake Superior Basin Watershed Study was designed to test a watershed classification scheme as a means to explain natural variation in watershed condition, identify causes of biological impairment, and predict relative sensitivity and resilience of



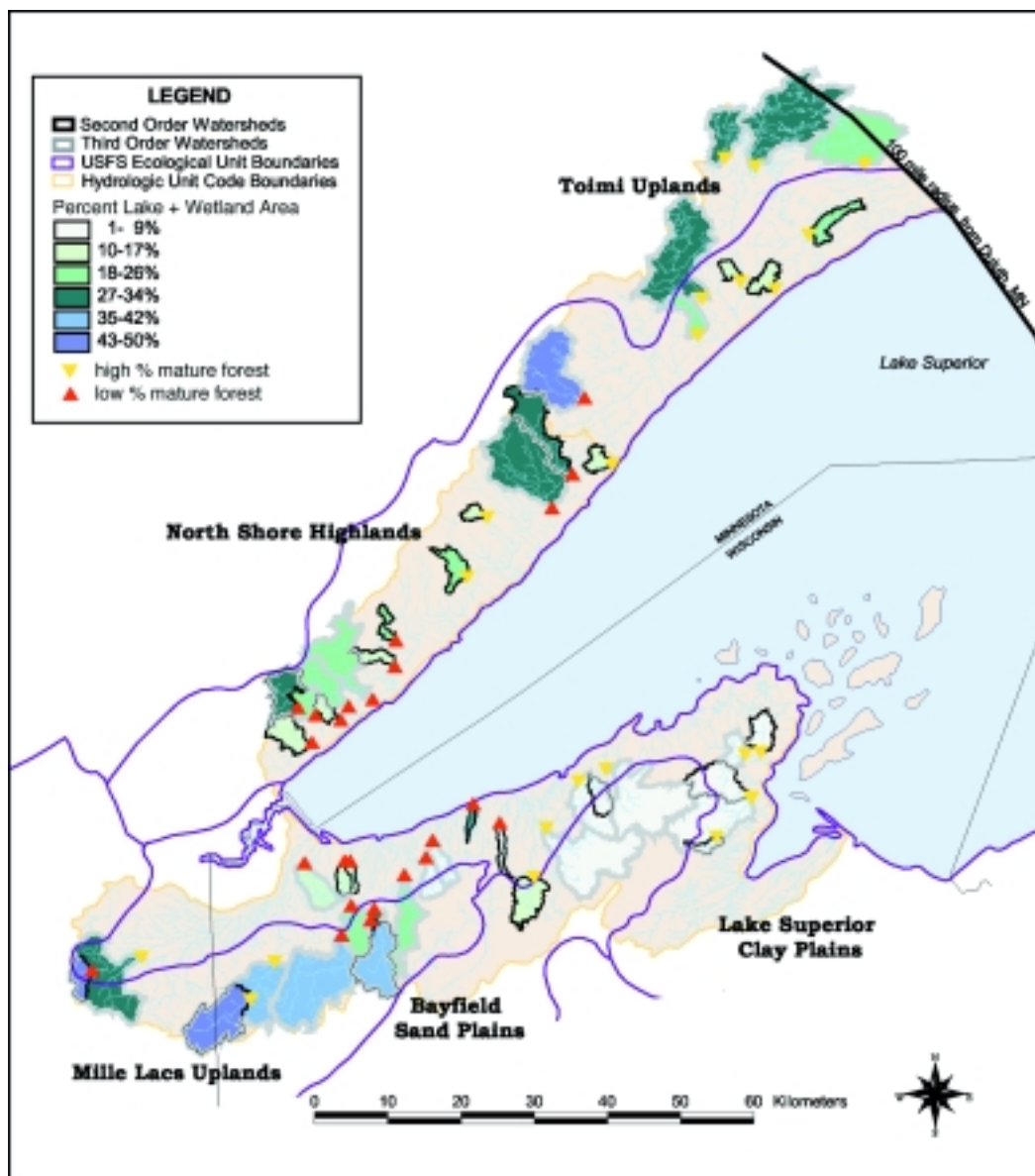
Bi-raphe diatoms serve as one community-level indicator of clean sediment impacts.

aquatic systems to nonpoint source pollution. This project will show how watershed classification systems can be used to extrapolate the results of watershed assessments among watersheds and regions. This will lead to improved efficiencies in monitoring watershed condition, estimating Total Maximum Daily Load (TMDL) of contaminants, and targeting watersheds for restoration. Further, scientists with the Lake Superior Basin Watershed Study are developing diagnostic signatures associated with the causes of biological impairment at both community and watershed levels.

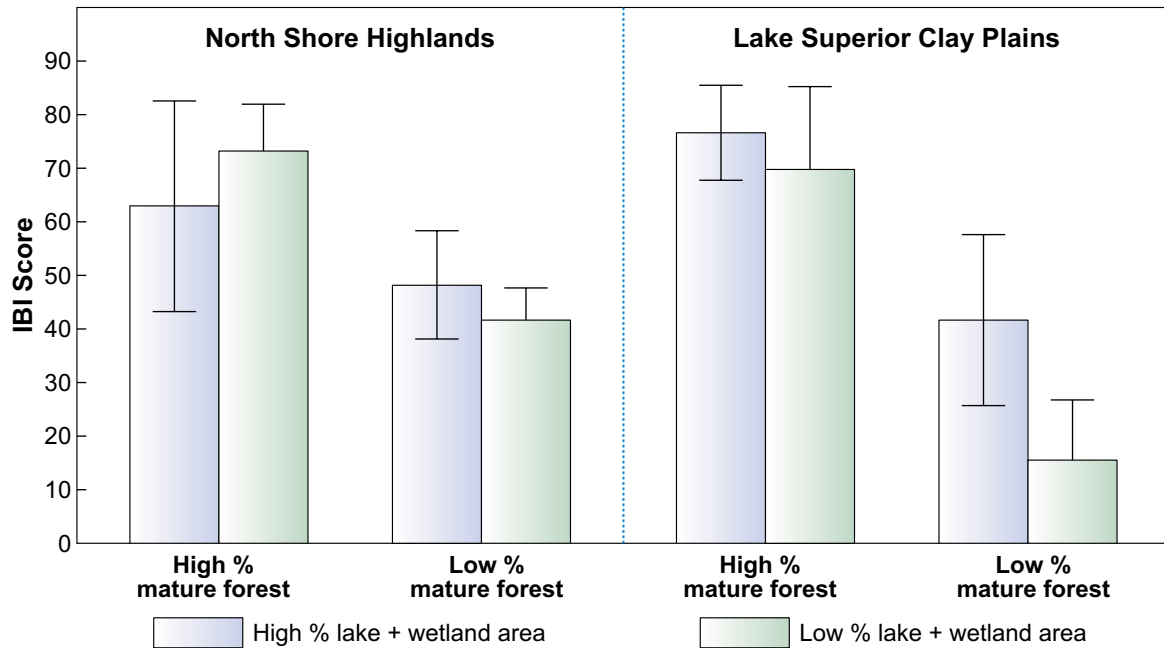
Two series of comparative watershed studies were conducted on two regions that varied with respect to soil type and hydrologic properties. The North Shore Highlands are characterized by a relatively steep elevation gradient with thin soils and bedrock-dominated streams. The Lake Superior Clay Plains have a lesser elevation gradient and thicker, erodible clay soils. Within each region, watersheds were classified by low *versus* high watershed storage

capacity (percent area occupied by lakes and wetlands), and, within storage capacity category, by low *versus* high mature forest cover. Measures of habitat quality and fish community health were lowest within the Lake Superior Clay Plains, in watersheds with low storage capacity and low mature forest cover. (See graph facing page.)

Watersheds around one portion of Lake Superior, showing distinct differences between the North Shore Highlands and the Lake Superior Clay Plains with respect to percent mature forest and percent watershed storage capacity (lake plus wetland area).



Index of biotic integrity (IBI) scores measure fish community health.



Fish community index of biotic integrity (IBI) scores by watershed class for north and south shore hydrogeomorphic units, high and low % mature forest, and high and low % watershed storage (lake + wetland area). IBI scores are significantly higher ($p < 0.001$) in high % mature forest class watersheds.

EPA recognizes that it is cost-prohibitive to monitor all the nation's waters. Water quality and sediment content vary at different locations of lakes, streams, rivers, estuaries, and wetlands, and in different bodies of water in a region. When taking water and sediment samples, a major challenge is to obtain samples from the different areas of a lake, stream, river, estuary, or wetland so that the results are representative of the entire region. Historically, samples of aquatic systems have been collected based on "point source" problems, and these samples are typically not representative of the waters in a region. To avoid this type of bias, the sampling procedures used in the Coastal 2000 Initiative and the Western Pilot are based on statistically random sampling procedures. By using a random sampling design to select a subset of waters for monitoring within an area, the findings for these waters can then be extrapolated to the unmonitored waters in the area. The Lake Superior Basin study added an additional innovation; it was the first regional test of a *stratified* random sampling design to assess ecological indicators at a landscape scale.





The primary responsibility of the Environmental Protection Agency (EPA) is to protect human health and the environment. In doing so, EPA uses risk assessments to identify and characterize environmentally related human health problems. Many uncertainties exist in the risk-assessment process due to the complex relationship between exposure to a chemical in the environment, distribution of the chemical in the body, and the toxic or adverse effects of the chemical in individual humans. Current risk-assessment procedures rely heavily on default assumptions that were made in the absence of relevant scientific data. For example, EPA often makes risk-assessment decisions based on information derived from animal models.

on such default assumptions by providing a mechanistically based understanding of toxicity and the factors associated with susceptibility. NHEERL is also developing models to account for exposure scenarios that differ with respect to media (air, water, or soil), route of exposure (inhalation, ingestion, or skin absorption), temporal dimensions, and other factors.



Sound Science

human health protection

In the laboratory, animals have historically been exposed to conditions that do not precisely mimic the exposure conditions to which humans are subjected in the environment. Three assumptions operating in this scenario are (1) variation in exposure conditions does not affect the validity of the results, (2) sensitivity to the chemical is similar for humans and the animal model, and (3) detoxification of the chemical is similar for humans and the animal model. These and other assumptions introduce uncertainty into the process by which risk assessors attempt to determine if a chemical poses a threat to human health. One important objective of NHEERL is to reduce reliance

One of the greatest uncertainties in the risk-assessment process is the fact that individuals differ in their response to chemical exposure. Further, certain identifiable subpopulations within the general population may be differentially susceptible to a given exposure level. For example, children may be more sensitive than adults to the toxic effects of some chemicals.

Research on susceptible subpopulations at NHEERL is based on the hypothesis that variability in response to environmental toxins is due in part to biological variability.

“One of the greatest uncertainties in the risk-assessment process is the fact that individuals differ in their response to chemical exposure.”

This variability depends on internal factors (gender, race, age, and specific genetic factors) and on external factors (prior exposures, pre-existing disease, activity level, nutrition, stress, licit or illicit drug use, cigarette smoking, alcohol use, or socioeconomic status). A major emphasis of the program on human health protection is to understand more clearly how variability in human susceptibility alters response to chemical exposures.

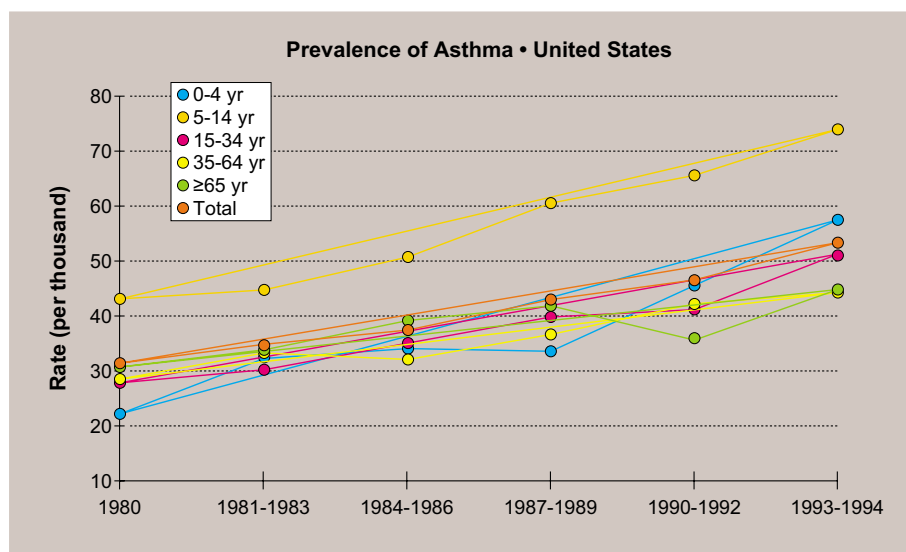
AIR POLLUTION AND ASTHMA

In the United States, the prevalence of allergic asthma has been increasing steadily since 1980, especially among adolescents and children 14 years of age or younger. NHEERL scientists are using human

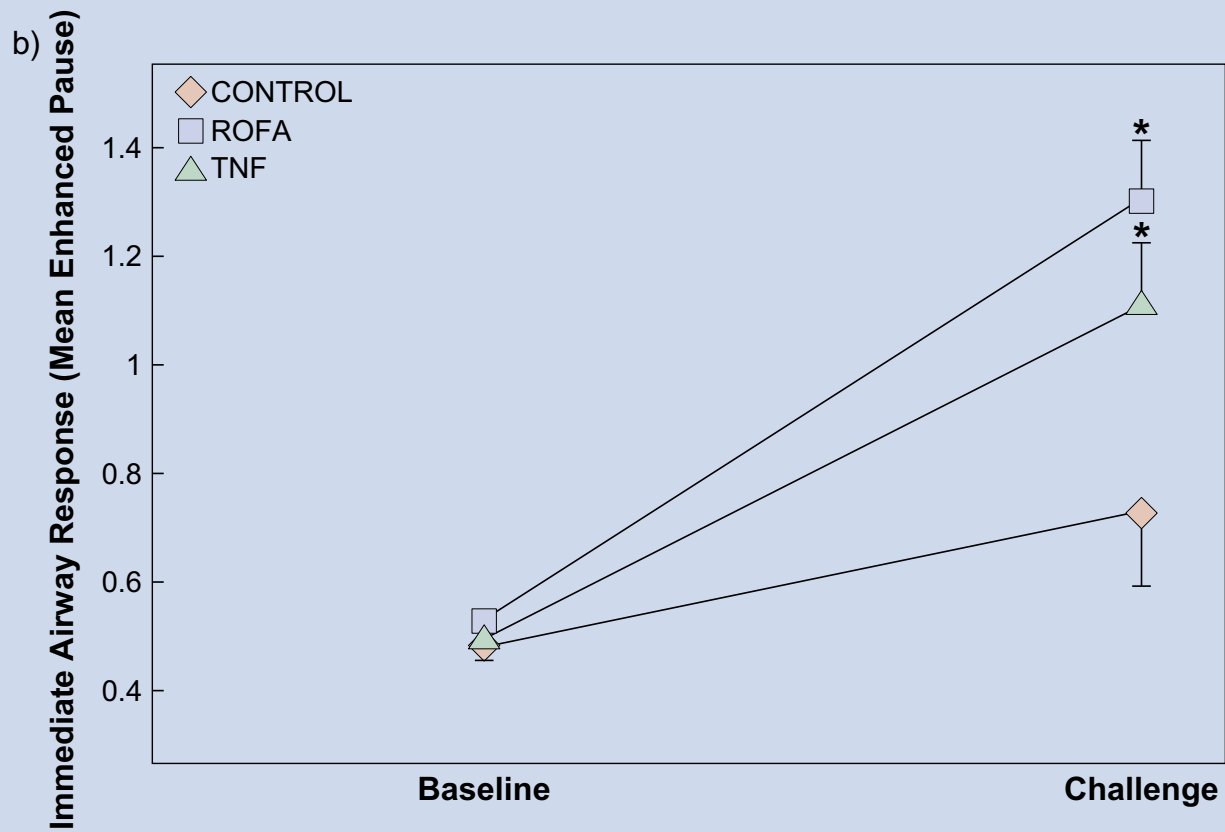
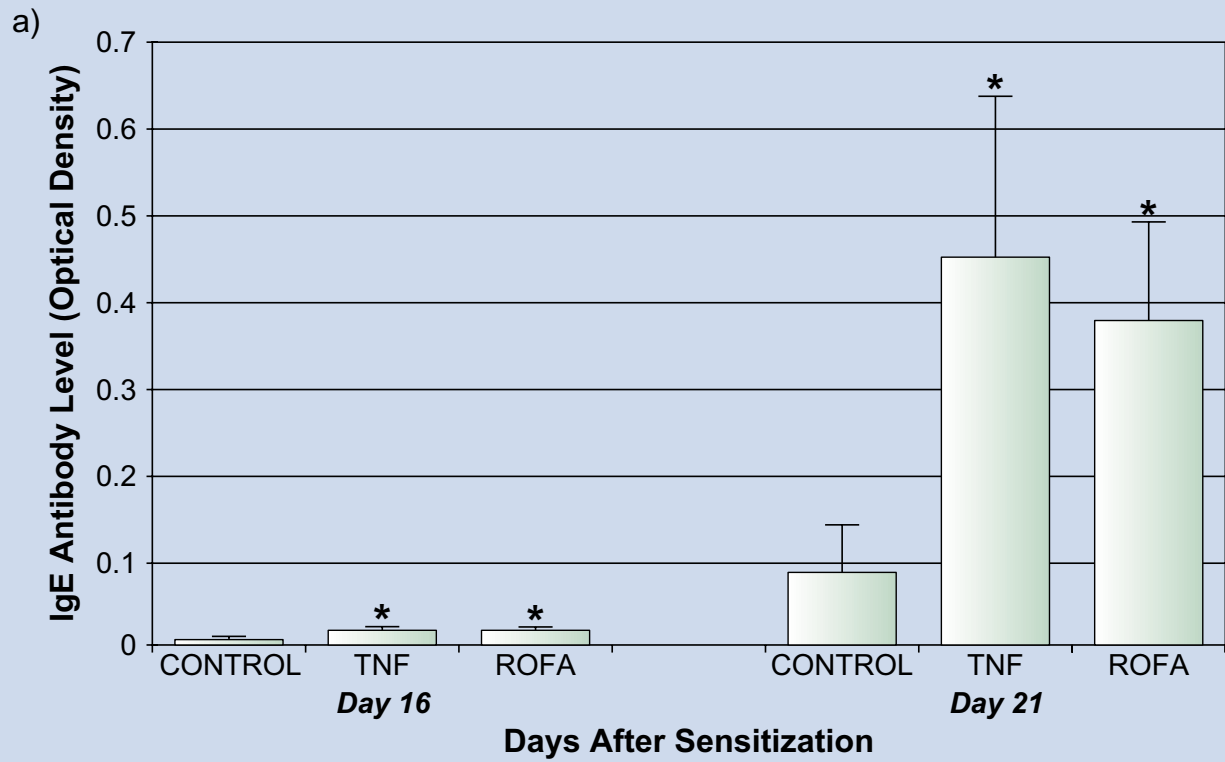
volunteers, epidemiologic studies, and animal models to study how air pollution contributes to the development and expression of allergic asthma. NHEERL scientists have developed mouse and rat models that exhibit many of the characteristics of human allergic asthma. These animal models are being used to

- test the hypothesis that air pollutants and other environmental agents enhance the development of allergic diseases.
- identify specific agents that trigger allergic reactions.
- identify biological parameters that can be used to rank the relative potency of triggering agents.
- study the mechanisms by which air pollutants exacerbate the symptoms of allergic asthma.

Using a rat model of allergic asthma, NHEERL researchers discovered that exposure to residual oil fly ash (ROFA), a product of fuel oil combustion, increased the allergic sensitivity to dust-mite allergen. Allergic sensitivity is determined by measuring dust-mite allergen-specific IgE antibody levels and immediate bronchoconstriction response. (See graph on facing page.)



The prevalence of asthma in the United States has been increasing since 1980, especially among children and youth 14 years of age and younger.



Exposure to residual oil fly ash (ROFA) stimulates the release of the pro-inflammatory cytokine TNF- α . Exposure to either ROFA or TNF- α enhances (a) IgE antibody and (b) immediate airway response to dust-mite allergen. These findings provide evidence that ROFA sensitization to dust-mite allergen is associated with an increase of inflammatory cytokines in the lungs.

“Research on susceptible subpopulations at NHEERL is based on the hypothesis that variability in response to environmental toxins is due to biological variability.”

A follow-up study determined that the metal components of ROFA cause the allergic sensitization. These asthma studies are an excellent example of how NHEERL researchers are discovering the physiological mechanisms underlying the relationship between environmental contaminants and human health.

NHEERL's studies in human volunteers that were completed in Fiscal Year 2000 confirmed that asthmatics and nonasthmatics exhibit different inflammatory responses in the lung when exposed to

air pollutants. Compared to nonasthmatics, asthmatics responded to lower concentrations of air pollutants and released larger quantities of a different set of inflammatory proteins. Information from these and other studies provides risk assessors with a very important mechanistic basis for considering sensitive subpopulations in the risk-assessment process. Other diseases being studied for their potential to increase susceptibility to toxin exposure include diabetes, cardiovascular disease, and other pulmonary conditions.



Preparing DNA samples for agarose gel electrophoresis to look for genetic polymorphism.



NHEERL scientist analyzing DNA for the presence of base substitution genetic polymorphism.

GENETIC POLYMORPHISM AND SENSITIVITY TO TOXINS

Genetic polymorphism, the existence of multiple forms of a gene, is thought to be associated with increased risk of adverse health effects following exposure to some environmental contaminants. NHEERL research on arsenic metabolism supports the general hypothesis that variations in response to chemicals are associated with polymorphism in the genes responsible for chemical metabolism.

During animal studies, NHEERL scientists discovered variation in the rate at which individuals methylate arsenic metabolites. (Arsenic is detoxified in the body when its metabolites are methylated). More

importantly, the researchers found that the rate of metabolite methylation was associated with the rate at which arsenic toxicity developed. Animals that methylated arsenic metabolites slowly developed toxic signs earlier than animals that methylated arsenic metabolites rapidly. Based on this research, epidemiologic studies will be conducted at NHEERL to determine the role of genetic polymorphism in the toxicity of arsenic to humans. Ultimately, this research could lead to the identification of biomarkers—measurable physiological parameters—that would enable scientists to predict the likelihood of increased arsenic sensitivity among certain subpopulations.



Fathead minnows (*Pimephales promelas*)

Endocrine disrupting chemicals (EDCs) are exogenous chemical substances or mixtures that interfere with the production, release, transport, metabolism, binding, action, or elimination of the natural hormones of the body. EDCs may adversely affect individual organisms, progeny, populations, or subpopulations. A broad range of substances, including widely used pesticides and numerous industrial chemicals, have been identified as potential endocrine disruptors. Because of the potential scope of the problem, the possibility of serious effects on the health of populations, and the persistence of some EDCs in the environment, endocrine disruption is one of the six high-priority research areas identified in EPA's Office of Research and Development Strategic Plan.

Evaluating tissue samples for evidence of endocrine disruptor activity on reproductive development in the female rat.



Sound Science

endocrine disruptors

Regulatory control of endocrine disrupting chemicals falls under four legislative acts. The Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) provide EPA with the authority to evaluate the toxicity of industrial chemicals and pesticides. In addition, the Food Quality Protection Act (FQPA) and the Safe Drinking Water Act Amendments (SDWAA) of 1996 require the testing of pesticides and chemicals found in food and water to determine their “estrogenic or other endocrine effects in humans.”

In the evaluation of endocrine disruptor activity on pubertal development in the male rat, an NHEERL scientist uses a robotic pipettor to prepare serum samples for radioimmunoassay.



As a consequence of these legislative directives, the Endocrine Disruptor Screening and Testing Program (EDSTP) was established to develop standardized protocols for *in vivo* and *in vitro* assays to detect chemicals that affect estrogen, androgen, and thyroid function. NHEERL researchers have taken a lead role in developing many of these assays. In Fiscal Year 2000, NHEERL scientists have been involved in a number of projects related to this effort, including

- developing or examining various *in vitro* tests for identifying potential endocrine disruptor activity (for example, competitive binding assays and androgen/estrogen gene regulation tests).
- finalizing protocols for screening assays in male and female pubertal rats. These assays evaluate endocrine disruption from chemical exposure occurring during puberty, a developmentally sensitive life stage.
- working with the Organization of Economic and Community Development (OECD) and serving as the lead laboratory for the standardization and validation of an *in vivo* test for androgenic activity (the Hershberger Assay).



***In vitro* techniques being used to assess environmental contaminants for androgenic and/or antiandrogenic activity.**

Other NHEERL researchers have developed a short-term assay using the fathead minnow (*Pimephales promelas*) to evaluate the effects of chemicals on endocrine and reproductive function. The test starts with reproductively mature minnows and examines the effects of chemical exposure on hormone levels, gonadal status, reproductive behavior, secondary sexual characteristics, fecundity, egg fertility, and early embryonic development. This test is cost-effective and can be completed in a fraction of the time required for a traditional full life-cycle study.

In addition to developing screening assays, NHEERL scientists are studying the specific reproductive effects caused by endocrine disrupting chemicals and the mechanisms behind these reproductive effects. One active area of research at NHEERL is the link between chemical exposure and inflammation of the prostate (prostatitis) in male rats, which is mediated by altered prolactin levels. Prolactin is a pituitary hormone that regulates many functions, including brain development. In rats, prolactin provided in the dam's milk influences development of the brain area that ultimately regulates prolactin levels in the adult. A decrease in milk prolactin levels during critical brain development results in altered prolactin levels in the offspring. The long-term consequence is prostatitis when the young males mature into adults.

Atrazine is one of the chloro-S-triazine herbicides, the largest group of herbicides sold in the United States. NHEERL scientists discovered that atrazine acts on the pituitary gland of nursing rats, suppressing prolactin secretion in the milk. Male rats whose dams

were exposed to atrazine during the first nine days of lactation exhibited an increased incidence and severity of prostatitis when mature.

Neonatal exposure to other substances, including estrogen, can also alter prolactin secretion. Based on this observation, NHEERL researchers hypothesized that perinatal exposure to estrogenic compounds may increase the risk of prostatitis in male offspring by decreasing prolactin levels in the dam's milk. They exposed pregnant rats late in gestation and their pups during the first five days after birth to either the hormone 17beta-estradiol, the insecticide methoxychlor, or the pharmaceutical agent tamoxifen. As adults, the male rats exposed to these chemicals perinatally experienced a significantly higher incidence and severity of lateral prostate inflammation compared to controls. These and other studies demonstrate a previously unrecognized critical period of susceptibility to chemicals that affect the pituitary gland.

“NHEERL researchers hypothesized that perinatal exposure to estrogenic compounds may increase the risk of prostatitis.”

NHEERL scientists are also studying the mechanisms by which phthalates cause reproductive abnormalities in animals. One of the most widely used phthalates is di(2-ethylhexyl)phthalate (DEHP), a plasticizer used in toys, food wraps, cosmetics, vinyl floors, and medical products, including blood transfusion and

dialysis equipment. In previous studies, *in utero* exposure to DEHP, dibutyl phthalate (DBP), butylbenzyl phthalate (BBP), and di-isononyl phthalate (DINP) caused malformed sexual organs and disrupted androgen-dependent processes in male rat offspring. NHEERL studies of the antiandrogenic effects of DEHP and its major metabolite found that neither compound binds to the human androgen receptor. (Binding to the androgen receptor is a common antiandrogenic mechanism.) Other studies indicate that inhibition of testicular testosterone production is the most likely mechanism by which DEHP disrupts sexual differentiation. These and other studies identifying the specific mechanisms of toxicity provide a scientific basis for the risk assessment of human exposure to endocrine disrupting chemicals.

In rodent studies to identify chemicals that may alter endocrine function, NHEERL scientists found the following effects.

- Dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP), and di-isononyl phthalate (DINP) caused retained nipples, altered testis descent, vaginal pouch formation, and malformations of the penis, prostate, epididymis, and other reproductive tissues.
- Linuron caused atrophied testes, malformed penises and epididymides, retained nipples, and reduced anogenital distance.
- Ketoconazole caused delayed delivery and fetal death.
- Chloro-S-triazine herbicides (atrazine, simazine, cyanazine) disrupted hormonal control of ovarian cycles.

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